POPULATION GENETICS OF SELECTED WHITEBAIT SPECIES: *GALAXIAS MACULATUS* (JENYNS) AND *LOVETTIA SEALII* (JOHNSTON).

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Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any university and that, to the best of my knowledge, this thesis contains no copy of previously published material except where due reference is made in the text.

N. C. Pavuk

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Summary

Population Genetics of Selected Whitebait Species: *Galaxias maculatus* (Jenyns) and *Lovettia sealii* (Johnston).

Populations of diadromous *G. maculatus* and anadromous *L. sealii* fishes have been studied for genetic variation by cellulose acetate electrophoresis.

L. sealii is a small member of the family Aplochitonidae endemic to Tasmania. In contrast, *G. maculatus* a salmoniform fish of the Family Galaxiidae, is found throughout the southern temperate zone (Australia, New Zealand and South America). Together these species are the predominant components of Tasmanian whitebait.

A significant commercial fishery for Tasmanian whitebait began in 1941 and rapidly declined after 1947 probably due to overfishing (Blackburn, 1950), until its closure in 1974. Encouraging runs of whitebait in the early 1980s prompted a major study to investigate the potential yield and present status of this resource. The lack of data regarding the genetic structure of the species prompted the following study.

Eight populations of *G. maculatus* extending from Western Australia to New Zealand were investigated. Twenty seven samples of *L. sealii* representing thirteen different river populations from Tasmania were included to capture genetic data both within and between three successive spawning seasons. An analysis of the gene frequency data using Nei's genetic distance (D) and identity (I) , G-test and F-statistics indicated population substructuring in the two species. Morphometric and meristic analysis of *L. sealii* was also undertaken and supported the genetic data.

Within *G. maculatus*, the Western Australian population was genetically distinct primarily based on allele frequencies observed at the ADH locus. Three genetic pools of *G. maculatus* were identified which have zoo-geographic implications for this species. In particular they appear to confirm the potential of marine larval dispersal between Australia and New Zealand.

A clear genetic discontinuity was observed between north and south Tasmanian *L. sealii* primarily based on the allele frequency data at the PEPD locus. These findings were consistent with those of Blackburn (1950) based on differences in growth rate and pigmentation. However, many southern rivers were found to carry unique stocks. A major contributing factor to this genetic diversity is thought to be the prevailing local hydrographic conditions.

The results of this study have implications for the management of the recreational whitebait fishery which has re-opened in Tasmania and possibly the commercial whitebait fishery in New Zealand which is dominated by *G. maculatus*.

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Chapter 1

1.0 Introduction

1.1 General Aims

Large spring migrations of whitebait were observed in the 1980s by members of the Tasmanian public and, in particular, by retired whitebait fishermen (W. Fulton pers. comm.). The current study was initiated in response to mounting public and political pressure to re-open the whitebait fishery.

This study was undertaken as part of a Fishing Industry Research Trust Account (F.I.R.T.A.) project (85/52) to determine if the present whitebait populations in Tasmanian rivers could support any level of commercial or recreational fishing. If a sustainable yield could be identified, guidelines for management of the fishery consistent with species and stock conservation would be established. Also included was the possibility of implementing measures to protect populations and, if possible to assist the recovery of the fishery.

The two most important components of Tasmanian whitebait are the species *Lovettia sealii* (Johnston) and *Galaxias maculatus* (Jenyns). Other less important members of the whitebait runs include *Galaxias truttaceus* Valenciennes, *Galaxias brevipinnis* Gunther, *Galaxias cleaveri* Scott and *Retropinna tasmanica* McCulloch (Fulton, 1990). The extent to which *G. maculatus* and *L. sealii* occur as ecologically and geographically differentiated stocks in Tasmania is a matter of practical interest to fisheries managers, particularly when contemplating the re-opening of the commercial fishery. The identification of distinct genetic populations within the area would establish the need for stock conservation and possibly provide a tool for stock identification. Conversely, if regional populations could not be distinguished by genetic methods, there might be less need for managers to take population structure into account allowing for a broad scale management plan of the species.
This research had three major goals:

1. to establish the optimal conditions for the electrophoretic analysis of stocks of *Lovettia sealii* and *Galaxias maculatus*,
2. to obtain frozen tissue samples of each species from various localities throughout the species range and, analyze these samples for evidence of stock heterogeneity; and
3. if the existence of two or more genetically differentiated stocks was detected, to determine if possible the genetic characteristics and geographic boundaries of each stock.

1.2 *Lovettia sealii*

1.2.1 Taxonomic Background

Lovettia sealii (Johnston) was originally described as a species of Aplochiton (Johnston, 1883). McCulloch (1915) recognised its generic distinction from Aplochiton and established Lovettia. The genus was named in acknowledgement of the specimens

captured in the Derwent River, Hobart and preserved by Mr E. F. Lovett (McCulloch, 1915).

Jenyns (1842) placed Aplochiton in the family Salmonidae. In a comprehensive taxonomic review, McDowall (1971) considered Lovettia a member of the family Aplochitonidae which contained two genera: Aplochiton Jenyns with 2 species found in South America and Lovettia a monotypic genus found in Tasmania, Australia (McDowall, 1971). The species of Aplochiton recognised by McDowall (1971) were *A. zebra* Jenyns and *A. taeniatus* Jenyns. Both are found in Chile, Argentina, Tierra del Fuego and the Falkland Islands.

Features of the Aplochitonidae include: small to medium size fishes (up to 240 mm), urinogenital apertures on a papilla set in a post-anal depression (Aplochiton) or protruding (Lovettia), sexes similar (Aplochiton) or dimorphic (Lovettia), lacking in scales but with a lateral line. These and other features are detailed by Blackburn (1950) and McDowall (1971). The most important characteristic of Lovettia is the position of the urinogenital aperture which occurs in front of the origin of the anal fin in females while in the mature male, coelomic organs extend back only as far as the pelvic fins with the urinogenital and anal apertures found in the isthmus (McDowall, 1971).

The Aplochitonidae has a trans-Pacific range, with species in Tasmania and South America. Although sharing common ancestry, Lovettia and Aplochiton are still rather different and have diverged considerably from the common ancestor (McDowall, 1971). Gosline (1960) found the genera very different in morphology and suggested the formation of two separate families but refrained from formally doing so. To date the inclusion of the two genera in one family may be simply to avoid over-fragmentation and its trans-Pacific range may therefore be an artifact of taxonomic grouping. In his recent review, Begle (1991) proposed that the Galaxiinae, Aplochitoninae and Lovettiinae be incorporated in an expanded family Galaxiidae.

1.2.2 Life History

Lovettia sealii is a small, scaleless fish known only from Tasmanian waters of Australia. The majority of fish spawn only once, and live for just over one year. The only stages in the life history that have been observed in the wild are the mature adults as they ascend rivers to spawn, the eggs and very young larvae. There are no data available on the fate or distribution of fishes after they descend shortly after hatching until their ascent into tidal river reaches as mature adults (Blackburn, 1950).

Lovettia sealii are generally captured in Tasmanian rivers during their spawning season which extends from August to November. On the north coast of Tasmania the spawning season spans the months of August to October, while in the south of the state this species dominates the whitebait runs between September to November (Blackburn, 1950). The sighting of spawning adults varies according to differences in the time of entry of fish into the spawning grounds, the position of fishing sites on the rivers, precipitation in individual

watersheds and the volume of flow downstream (Blackburn, 1950). Floods have been observed to slow or completely halt the ascent of this species.

The overwhelming majority of specimens caught during the major study of Blackburn (1950) were reproductively mature individuals. Females had characteristically distended body cavities containing immature, opaque white eggs of about 0.4 mm in diameter ranging to large transparent eggs of 1.1 mm diameter and usually yellowish in colour. Mature males had obvious, white testes of 6-8 mm in length.

Blackburn (1950) observed eggs adhering in large numbers as single sheets to submerged logs, rocks, wooden pier beams and rock platforms below the low water level.

Blackburn (1950) considered that adult *Lovettia sealii* caught in any river were nearly always a single, year 1 age class. The actual age of indivdual fish may vary up to 3 months which reflects the duration of the spawning season and a difference in hatching times. Only a minute proportion (0.001%) of the population was represented by two year old fish; the majority of these fish were females. The two-year old fish are characteristically much larger than one year olds. Examination of otoliths confirmed the presence of some two-year old specimens and Blackburn (1950) suggested that one or two of the largest specimens he sampled may even have been 3 years old.

The sex ratio in samples of *Lovettia sealii* captured by Blackburn (1950) was observed to be highly variable. It varied from 10:1, male: female, in the Duck River, 3:1 in the Leven River and almost 1:1 in the Don River over several pooled catches. There was a general trend for males to predominate the catches in both the north and south of the state.

The pigmentation of *Lovettia sealii* varies from translucent to fish with large black spots along the posterior of the back, lateral line and head. Blackburn (1950) devised a five stage pigmentation scale for this species. He established that successive gonad-maturity stages are characterised by increased pigmentation. The males are found to be generally darker than the females. Pigmentation was also used by Blackburn (1950) to separate northern and southern populations of *Lovettia sealii*. He noted that the specimens caught in southern Tasmania were typically more pigmented than the northern fish of the same sex and stage of maturity.

It is generally accepted that most fish of this species die shortly after spawning. This was supported in the current study by the observed poor condition of spent fish, which were very lean. Annectodal evidence from fishermen relate the occasional appearance of vast numbers of dead *Lovettia sealii* washed up on northern coastal beaches at the end of the spawning season. These fish have been used as a garden fertilizer by locals (Mr L. Simms, pers. comm.).

Prior to Blackburn's study there was a belief that there were two populations of *Lovettia sealii* in Tasmania, one in the north and another in the south, and that these populations would need to be managed separately. Blackburn (1950) investigated variation in the mean

number of vertebrae in an attempt to find evidence for discrete northern and southern populations of *L. sealii*. A statistical analysis of this data did not reveal any significant difference between northern and southern representatives. The more extensive pigmentation in southern fish and the greater growth rate of northern fish were thought to be the key in distinguishing northern and southern fish. The mean length of the northern fish was greater than that of the southern material of the same sex (Blackburn, 1950). The difference between mean female and male lengths was also greater in the north than in the south. The southern *L. sealii* were typically more pigmented. The northern population was also considered homogeneous because the decline in abundance of *L. sealii* in this area in 1948 affected all northern rivers. However, in a major field study conducted 1985-1988, Fulton *et al.*(1988) found that the average size of fish was not a reliable predictor of population substructuring. Size was related both to sex and time of capture. Detailed investigations of several rivers found that the size of fish from the Derwent River in the south exceeded specimens collected from both the Mersey and Rubicon Rivers in the north. However, fish caught in the far southern rivers such as the Catamaran and Lune were consistently smaller than those caught elsewhere.

1.3 *Galaxias maculatus*

1.3.1 Distribution

Galaxias maculatus is a member of the family Galaxiidae. This family is of widespread interest partly because of its relationships within the salmoniform fishes (McDowall, 1969; Rosen, 1974), and partly due to its zoogeography (McDowall, 1970, 1972, 1975, 1978; Rosen, 1974). There are currently 36 species recognised within the Galaxiidae. Twenty species occur in Australia (18 of which are endemic), 13 in New Zealand (11 endemics), 4 in South America (3 endemics), and one species in each of South Africa and New Caledonia. Tasmania possesses 15 of the 20 Australian galaxiid species and 10 of these are endemic to the island (McDowall and Frankenberg, 1981). The proposed extension of the family Galaxiidae (Begle, 1991) referred to earlier is yet to be ratified and therefore the historical nomenclature has been used in this study.

Galaxias maculatus (Jenyns) is a small migratory freshwater fish common in most New Zealand lowland streams and known also from the Australian mainland, Tasmania, South America, and the Falkland Islands. Scott (1962) gave the distribution of *G. maculatus* in Australia as "coastal streams of South Australia, Victoria, New South Wales, southern Queensland, and Tasmania". In New Zealand it is present throughout the coastal regions of both the North and South Islands. The distribution in South America is not well documented, but Regan (1905) gave the range as follows: Tierra del Fuego, Patagonia, Chile, and the Falkland Islands. In Chile it is found north of Valparaiso.

1.3.2 Life History

Benzie (1961) and McDowall (1968) have both completed an extensive review of the biology of *G. maculatus*. The following is a brief summary of the life history.

After hatching on the banks of stream estuaries larvae are carried out to sea where they live for about 6 months. Little is known of the life history of *G. maculatus* from hatching to migration into freshwater. The juveniles migrate back into the coastal rivers, where they grow and mature. It is during this migration that young *G. maculatus* are captured as part of the whitebait runs. *Galaxias maculatus* grows to about 180 mm and probably has a maximum longevity of about three years. This species normally matures during its first summer in fresh water and breeds in the subsequent autumn, but a few maiden spawners may be 2 or 3 years old.

Downstream breeding migrations occur before the spring tides. These involve the movement of adult fish from middle river reaches which they inhabit as juveniles, into estuaries to spawn. Breeding has been recorded from September until June, but is usually in March to May. The reproductive organs of this species are typical for salmoniform fishes. The gonads usually mature between February and May. Spawning takes place in the tidal estuaries, typically on flat grassy banks exposed at all times of the tidal cycle except the high spring tides.

The egg of this species is small, about 1 mm in diameter, and spherical with numerous oil globules, and almost colourless. The eggs are sticky on extrusion and are deposited among estuarine bank vegetation at high spring tides. Fecundity varies widely, from 175 to 13,500 eggs. The eggs usually develop within two weeks of spawning and hatch at the next spring tide cycle.

Populations with this life history are well documented in Australia and New Zealand but although the marine-living juveniles have been found in South America, the estuarine, tide-controlled spawning as known in Australia and New Zealand has not yet been described for that region.

1.3.3 An evolutionary perpective of the Galaxiidae

There has been much discussion about how galaxiids attained their very wide distribution in the southern hemisphere. McDowall (1983) suggested that the current distribution of the family is mainly due to marine dispersal and that freshwater species within this family have evolved from the dispersal of diadromous marine ancestors. In contrast, Campos (1974) maintains that vicariant events resulting from the Mesozoic breakup of Gondwanaland have determined the distribution of the Galaxiid family. Croizat *et al.* (1974) regarded these fishes as part of the pan-austral Gondwanaland biota, and Rosen (1974) believed their distribution to be connected with the fragmentation of Gondwanaland in the Mesozoic.

Controversy also continues over the probable nature of the galaxiid ancestor and whether it was predominantly saltwater or freshwater. At least six members of the family including *G. maculatus* are diadromous, spending about 6 months in the sea as larvae and post-larvae (McDowall, 1970). Although larval fish are thought to remain in coastal seas there are reports of them being found in the open ocean. McDowall *et al.*(1975) recorded galaxiid larvae in the southern Pacific Ocean 700 km east of New Zealand. Osteological

studies suggest that the diadromous species are amongst the more primitive members, and that the wholly freshwater species tend to be more derived in character (McDowall, 1970). Examination of distribution patterns shows that diadromous species are also more widely and generally distributed than the wholly freshwater species (McDowall, 1973). Distribution patterns are consistent with the view that euryhaline species which live in the sea for some months can, and do disperse. The sea-going species being at least as primitive as any, McDowall (1971) considered it reasonable to assume that early dispersal of galaxiid fishes could have been through the sea, thus freeing galaxiid dispersal and evolution from the constraints applied by Croziat (1975) where existing patterns were thought to be established by the fragmentation of Gondwanaland in the Mesozoic. As a result it is no longer necessary to assume that *Galaxias maculatus* has not undergone any phyletic evolution since the Cretaceous, in its geographic isolates in Australia, New Zealand and South America.

It is of considerable significance that, at least in the southern temperate zone, the land masses originating in Gondwanaland in the Mesozoic times now lie along the path along which existing wind and ocean currents promote dispersal. North-south lying continents and island archipelagos intersect the strongly east-west flow of wind and water in the temperate and subantactic of the southern hemisphere (roaring forties wind and the west wind ocean drift current).

The Galaxiidae and southern freshwater lampreys (sub-family Geotrinae) (Potter and Strahan, 1968) have a trans-Pacific range, with species in Tasmania and southern South America. *Galaxias maculatus* and *Geotria australis* Gray are both found in Australia, New Zealand, and South America and both have marine stages in their life histories. The fact that there are species common to these areas suggests that any fragmentation of a southern continent must have been far too early in time to permit the use of continental drift to explain the range of these species. McDowall (1971) suggested that transoceanic dispersal of these species.

1.4 History of the Whitebait Fishery

The history of the fishery for whitebait dates back to at least the early 1930s. However, significant commercial fisheries for the Tasmanian whitebait, *Lovettia sealii* began in 1941 in southern Tasmania, and in 1943 in the north of the state. The Tasmanian Fisheries Division introduced a licence system for fishermen in 1944. When the Tasmanian canning industry began to develop in the late 1930s it faced difficulties obtaining large and regular supplies of fish. As whitebait were known to be abundant and easily caught in certain rivers, it was natural that they should attract the attention of the canners. The whitebait fishery then grew rapidly in importance with a peak catch in 1947 of approximately 484 000 kg. Whitebait was particulary valuable to the canners as the fish were available at a time of year when other species were scarce. However, the catch per unit effort for individual fisherman during this period had already declined and by 1949 returns had fallen to under 50 000 kg (Blackburn, 1950).

A major study of the fishery by the CSIRO was commenced in 1945. The findings of this study, reported to both the Tasmanian Fisheries Division and the Tasmanian Sea Fisheries Advisory Board in 1949, recommended the complete closure of the season in the north of the state in that year and implementation of future annual quotas. The season re-opened in 1950 but the catches never approached the prescribed quotas. Deregulation of the fishery occurred in 1957, despite the catches for 1956 and 1957 being the lowest recorded since the fishery began. Control of the fishery was transferred to the Inland Fisheries Commission in 1965. During the following 9 years no attempt was made to arrest the decline in stocks and the fishery was finally closed by regulation in 1974 (Fulton, 1984).

At its peak, whitebaiting provided direct, part, or full-time seasonal employment for about 230 persons. Numbers declined to about 65 by 1960 whilst only 21 fisherman participated in the 1973 season. Despite its legal closure, an illicit fishery has continued for whitebait and some Tasmanian poachers received \$15-20.kg^{-1} for whitebait during 1985-1987. In New Zealand in 1985 the fishermen received \$23-27.kg^{-1} off the river whilst the fish retailed in Christchurch at \$45.kg^{-1} for galaxiid whitebait. This would value the average catch for all years of the Tasmanian fishery at approximately \$2.5 x10^{6} (Fulton, 1984).

1.4.1 Species compositon of Whitebait

There are six species commonly found in the whitebait runs in Tasmania:

Lovettia sealii Johnston	Tasmanian whitebait
Galaxias maculatus (Jenyns)	Jollytail
Galaxias truttaceus Valenciennes	Spotted galaxias
Galaxias brevipinnis Gunther	Climbing galaxias
Galaxias cleaveri Scott	Tasmanian mudfish
Retropinna tasmanica McCulloch	Tasmanian smelt

The four Galaxias species noted here also occur outside Tasmania and the jollytail is the predominant fish in the whitebait fishery of New Zealand. The Tasmanian whitebait and smelt are both endemic to Tasmania. A number of other migratory species also occur, to a minor degree in the catches, in particular the Australian grayling, *Prototroctes maraena* Gunther.

Fulton (1984) has also noted the historical change in contribution of *L. sealii* in the whitebait runs. In the late 1940s 95% of the runs were composed of *L. sealii;* this had fallen to 25% by 1965. He concluded that this reflected the reduction in abundance of *L. sealii* and not an increase in other species. Similarly Blackburn (1950) considered that the decline in stocks observed in the late 1940s and 50s was probably due to the depletion of the resource and not due to natural fluctuations. He considered the following observations to be pertinent:

1. the fishery operated almost exclusively upon the spawning fish and especially upon those which had not yet spawned;
2. the intensity of fishing had increased continuously and rapidly from year to year; and

3. the decline in abundance as measured by weight of fish was evident in 1947, and the decline in numbers probably earlier, in 1946 or even 1945.

If overfishing did occur it was by the excessive removal of parent fish, resulting in diminished reproduction remembering that, in *L. sealii,* only a minute proportion of the stock are two-year olds (0.001%) (Blackburn, 1950).

In northern rivers *L. sealii* is the dominant species in the runs from late August to October. *Galaxias maculatus* runs extended over a longer period whilst the runs of the other *Galaxias spp.* are of shorter duration. Details of the species composition and timing of whitebait runs has been extensively reviewed by Fulton *et al.* (1986) in an interim report of the FIRTA study.

1.5 Stock Identity and Population Genetics

1.5.1 Protein Electrophoresis

Electrophoresis was developed as an analytical technique for chemical and biological research (Richardson *et al.*, 1986). Since the mid-1960s multilocus protein electrophoresis had been the most widely employed molecular tool to assay genetic distances between species and a method for determining within-population variability in allozymes. This technique relies on the movement of charged particles (protein enzymes) in an electric field. This results in the spatial separation of the different enzymes in the support medium (gel matrix). Most proteins that are studied are enzymes because it is easy to visualise activities of specific enzymes following electrophoresis with histochemical stains. The localisation of an enzyme's activity in a gel has been called the "isozyme method". Isozyme refers to different distinguishable molecules found in the same organism which catalyze the same reaction. Allozyme commonly refers to the electrophoretic expression of allelic proteins at a particular locus. Details of enzyme-stain reactions are reviewed in Richardson *et al.* (1986). The banding pattern observed for an individual will contain information on that individual's genotype with respect to the locus (loci) coding for that particular protein (Ryman and Utter, 1987).

With the advance of gene isolation, cloning and nucleotide sequence determination, Avise and Aquadro (1987) considered that the era in which protein electrophoresis was a "state-of-the-art" survey technique is rapidly coming to an end. However, Ryman and Utter (1987) contend that it will remain a valuable tool for fish population genetics because it can generate large volumes of reliable genotypic and allele frequency data quickly and at relatively low cost. Also in its favour, protein electrophoresis is a simple technique which has been widely used to study genetic variation in populations. The primary goal of most genetic studies is to describe the pattern of genetic exchange and isolation among geographic units. The pattern of genetic divergence in natural populations results from the action of three basic evolutionary forces: migration, genetic drift and natural selection (Allendorf and Phelps, 1981). It has proved particularly useful because only small tissue samples are required from each individual and large numbers of individuals can be screened in a short time (Selander, 1976).

As reviewed by Ryman and Utter (1987) and Smith (1990) there are limitations to the information that can be obtained by electrophoresis at protein coding loci. The information needed in population genetics relates to base sequences of DNA studied either directly or indirectly. The amino acid substitutions of proteins detected by electrophoresis are indirect reflections of the actual base substitutions in base sequences. All base substitutions do not necessarily result in changes of amino acids nor do all changes result in protein changes that are electrophoretically detectable. Lewontin (1974) estimated that only a third of the amino acid substitutions are detected under standard electrophoretic conditions.

Large amounts of genotype and allele frequency data have been generated for numerous fish species. Many of the studies have shown that most species are subdivided into more or less distinct units that differ genetically from each other. The existence of such structure is a matter of major importance for management and conservation of genetic resources. An appreciation of the forces leading to appearance or disappearance of substructure and the time scales over which these phenomena take place is needed for interpreting data and estimating the impact of man's activities on existing population structure (Chakraborty and Leimar, 1987).

For example, genetic differentiation is now recognised as an important factor in the management of fish species such as the anadromous salmonids (Simon and Larkin, 1972). Because of their migratory behaviour and homing tendencies, differences in these species lies somewhere between the extremes of freshwater fish species, where extreme restriction of gene flow often leads to extensive differentiation between populations (Svardson, 1979), and marine species, where physical barriers to gene flow are often absent and differences are often smaller (Carscadden and Mirsa, 1980).

1.5.2 Population Models

Theoretical models of the evolution of a subdivided population have been developed for a number of idealised situations (Ryman and Utter, 1987). In 1943, Wright introduced the "island model". The name was derived from the proposition that a population occupied a group of islands with a subpopulation on each island and various amounts of gene flow between them. This model assumed that all subpopulations were randomly mating and of equal size. The rigid assumptions of the island model are often not realistic and have been modified by numerous workers e.g. Nei (1975) and Slatkin (1985). The island model, stepping stone, and the isolation by distance models, which are reviewed by Ryman and Utter (1987); all assume a population structure with one level, i.e. subpopulations, within the total. More complicated hierarchical models have been proposed which allow for a total population being composed of major subpopulations each composed of further subpopulations. The multitude of available models can act as a reference point when embarking on an electrophorectic study to help elucidate the population structure of the selected species. For example, Richardson (1983) used the isolation by distance model to explain the stock structure of skipjack tuna (*Katsuwonus pelamis)*.

When attempting to characterise the amount and distribution of genetic variation in a species, by far the most important consideration is to obtain genetic information form a

sufficiently wide range of subunits at different hierarchical levels. No method of analysis is universally best at describing the genetic structure of a species based on limited data. A given estimation procedure always contains idealised assumptions concerning the true nature of the genetic structure; these assumptions may or may not be valid for a particular species. Conversely, most methods are acceptable if sufficient data are available. It is probably more important for an investigator to be aware of the limitations that are due to an incomplete sampling of the species than to use a particular method of analysis (Utter *et al.*, 1980).

With limitations, protein electrophoresis is a powerful and simple technique for examining genetic variation in natural populations. From a practical perspective the genetic population structure of a species allows its distibution to be determined. Subsequent harvesting of the species can then be managed to preserve the genetic integrity of the resource.

1.5.3 Stock Concept

The Stock Concept International Symposium (STOCS) held in Ontario in 1980 thoroughly reviewed both the origins of the term "stock" (Booke, 1981) and the application of the stock concept to the regulation of fisheries (McDonald, 1981; Saunders, 1981) and prospects and strategies for the preservation of gene pools.

The term "stock" in this study will be used in the manner as defined by Booke (1981). The "genotypic" stock is characterised by a population of fish maintaining and sustaining Hardy-Weinberg equilibrium.

Chapter 2

2.0 Materials and Methods

2.1 Pilot study

A pilot study was undertaken at the Evolutionary Biology Unit of the South Australian Museum to assess if informative polymorphic loci could be detected in small samples of both *L. sealii* and *G. maculatus*. Samples of adult *L. sealii* (N=8) from each of the following five sites (Fig.2.1): Derwent River, Huon River, Rubicon River, Lune River, Inglis River and ten larval *G. maculatus* from each of four sites around Tasmania (Fig. 2.1): Great Forester River, Duck River, Prosser River, Derwent River were examined at a minimum of 30 enzyme systems.

Dr Mark Adams of the Evolutionary Biology Unit concluded that a large number of suitable enzyme markers were available to study the population structure of *L. sealii*. The following enzymes were considered most suitable for further examination: aGPD, GOT2, PGD, LDH2, PEPA and PGK. Although the electrophorectic screening was based on a very small sample, the early results suggested that a larger survey may yield valuable data and help elucidate the population structure of this species.

In *G.maculatus* 6 polymorphic loci were considered suitable for more detailed investigation: *ADA, FUM, PGD, PGM, GPI2* and *GPD*.

As a result of the pilot study it was determined that useful information regarding population structure may yet be gained from a larger and more detailed sampling, particularly in the case of *L. sealii*. A full listing of the enzymes systems that were investigated in both species are given in Table 2.1.

Table 2.1 Enzymes Investigated in Whitebait species

G. maculatus		
Enzyme	**Abbreviation**	**Enzyme Commission No.**
Adenosine deaminase	ADA	3.5.4.4
Peptidase	PEPD	3.4.11.
Phosphogluconate dehydrogenase	PGD	1.1.1.44
Phosphoglucomutase	PGM	5.4.2.2
Glucose phosphate isomerase	GPI2	5.3.1.9
Glutamate pyruvate transaminase	GPT	2.6.1.2
L. sealii		
Enzyme	**Abbreviation**	**Enzyme Commission No.**
Aspartate amino transferase	GOT1, GOT2	2.6.1.1
Glucose phosphate dehydrogenase	G6PD	1.1.1.49
Glycerol phosphate dehydrogenase	aGPD	1.1.1.8
Lactate dehydrogenase	LDH2	1.1.1.27
Mannose phosphate dehydrogenase	MPI	5.3.1.8
Phosphogluconate dehydrogenase	PGD	1.1.1.44

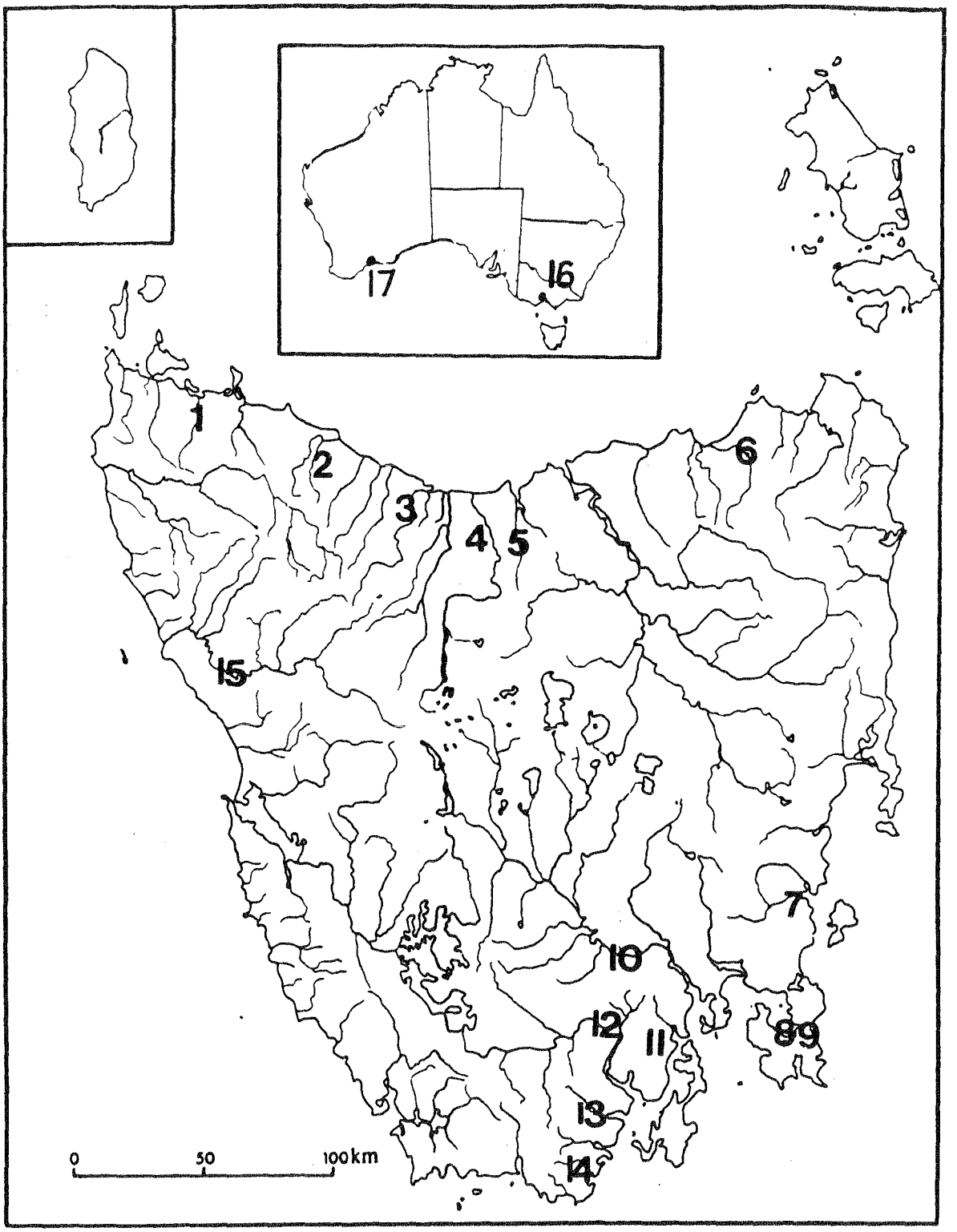


Figure 2.1 Location of sample sites for *L. sealii* and *G. maculatus*

Key

1	Duck River
2	Inglis River
3	Leven River
4	Mersey River
5	Rubicon River
6	Great Forester River
7	Prosser River
8	Allen's Creek
9	Parson's Bay Creek
10	Derwent River
11	Northwest Bay River
12	Huon River
13	Lune River
14	Catamaran River
15	Pieman River
16	Barwon River, VIC
17	Esperance River, WA

2.2 Main study

With the completion of the pilot study the following program was undertaken between 1985 and 1987 to determine the population genetic structure of *L. sealii* and *G. maculatus* .

2.2.1 Field Collection

Samples of reproductively mature *L. sealii* were collected over the known species range from 13 sites in Tasmania (Fig. 2.1). These included previously important centres of commercial fishing activity (Mersey, Rubicon, Derwent and Huon Rivers) prior to the closure of the fishery in 1974. Replicate samples were collected in the Mersey and Huon Rivers in 1985, and the Derwent River in 1986 in order to investigate any temporal variation in allele frequencies over a single tidal cycle. The Rubicon River sample collected in 1985 consisted of an equal ratio of male and female fish. It was used to investigate possible sex-linkage of loci. The sampling strategy also allowed for analysis of genetic variation within the Mersey River over the duration of the spawning season, and variation within and between multiple centres over three successive spawning seasons (1985-1988).

The following convention was used to catalogue all whitebait samples; River/Year of capture/Sample number during a single year. For example, Mersey/86/4 refers to the fourth sample caught in the Mersey River in 1986. Details of specimen number and collection site for *L. sealii* are given in Table 2.2.

Table 2.2. Collection of *L.sealii*

Collection Site	Grid Reference	Date	No.
Mersey River	41 44'S 146 24'E		
1		11.9.85	100
2		11.9.85	100
3		11.9.85	100
5		29.10.85	100
1		18.8.86	100
4		22.10.86	100
1		12.10.87	100
Rubicon River	41 44'S 146 34'E	11.9.85	100
Inglis River	41 18'S 145 42'E	24.11.85	100
Derwent River	43 15'S 147 05'E		
1		23.9.85	90
1		25.8.86	80
2		4.11.86	100
1		28.8.87	100
Parson's Bay Creek	43 04'S 147 15'E		
1		31.8.85	50
1		22.9.87	100
Allen's Creek	43 04'S 147 52'E	11.9.85	100
Lune River	43 25'S 146 25'E	10.10.85	100
Huon River	43 02'S 147 03'E		
1		31.10.85	100
2		31.10.85	100
1		12.11.86	100

Table 2.2 cont.

1		23.9.87	100
Leven River	41 41'S 146 04'E	2.10.86	100
Duck River	40 47'S 145 07'E	21.10.86	100
Catamaran River	43 06'S 146 25'E		
1		11.11.86	36
1		20.5.87	100
Pieman River	41 43'S 145 13'E	15.10.87	100
North West Bay River			
	43 04'S 147 15'E	4.9.87	32
		Total	2488

Larvae of *G. maculatus* were collected over a wide geographic range including eastern, south-eastern, north east and west coasts of Tasmania, southern Victoria, south- eastern Western Australia, and the east and west coasts of New Zealand's South Island. An indication of the stability of allele frequencies was investigated with replicate samples from the Derwent River over successive years. Details of specimen number and collection site for *G. maculatus* are shown in Table 2.3.

Table 2.3: Details of *G. maculatus* collection

Location	Grid Reference	Date	No.
Tasmania			
Derwent River	43 15'S 147 05'E	1986	100
Derwent River		1987	100
Duck River	40 47'S 145 07'E	1986	90
Great Forester River	41 01'S 147 25'E	1986	100
Prosser River	42 35'S 147 25'E	1986	95
New Zealand			
Ashley River	43 17'S 172 44'E	1986	100
Buller River	41 45'S 171 37'E	1986	65
Victoria			
Barwon River	38 08'S 144 23'E	1987	100
Western Australia			
Esperance River	33 50'S 121 56'E	1986	48
		Total	798

2.2.2 Method of Collection

Sampling of both species was done using traditional methods previously utilized by commercial fishermen. This involved the use of both "scoop" and "D-nets", as illustrated by Blackburn (1950) and built in accordance with the Inland Fisheries of Tasmania regulations which prevailed for the former fishery.

Modified mesh cages were used to capture samples of *L. sealii* in the Duck River. The cage had an internal funnel to prevent captured fish from exiting and external wing-like structures

to guide passing fish into the cage. The cage was positioned on the river bed at a depth that would not allow the use of conventional "scoop nets". In the Pieman River on the Tasmanian west coast these cages were set one on top of the other to allow the capture of specimens at different levels in the river.

Fishing for *L. sealii* was related to the tidal cycle. The fish did not generally begin to move into flowing water until the rising tide had slowed the downstream current. Nets were set facing downstream and lifted at regular intervals or when fish were seen. In northern rivers, nets were generally set along river banks where the fish would be caught as they moved upstream. In southern rivers fish were generally captured from a boat, by passing a scoop net directly through an advancing school. Light-framed, hand-held nets were also utilized to catch specimens.

2.2.3 Sample storage and preparation

Whole animal specimens were frozen in liquid nitrogen (-180°C) and stored until homogenates were prepared in the laboratory. Individual fish were thawed and the posterior third of the animal homogenised with an equal volume of cold homogenizing buffer (Appendix 2a). The tissue was mascerated with a teflon micropestle. The crude homogenate was centrifuged at 15000 G in an "Eppendorf" centrifuge for 7 minutes at 5°C. Resulting supernatant fluid was stored in 50µl aliquots in glass microcapillary tubes at -20°C for a maximum period of two weeks prior to electrophoresis. A minimum of ten tubes were stored per individual. Tubes were sealed with coloured plasticine which allowed the tracking of individuals at each locus, and the reliable identification of standards to be used on subsequent gels.

2.2.4 Cellulose acetate gel electrophoresis and histochemical staining

Tissue homogenates were subjected to cellulose acetate (Cellogel, Milan) electrophoresis modified after Richardson *et al.*(1986). Commercial preparations of "Cellogel" were stored in 30% methanol in an airtight container at 4°C . Gels were prepared for electrophoresis by blotting and transferring to the appropriate running buffer (Appendix 2b) for 30 min at 4°C.

The electrophoretic chamber and running buffer were precooled at 4°C for 30 minutes prior to electrophoresis. After equilibration the gels were blotted and positioned across the electrophoretic chamber containing approximately 700 ml of running buffer. The gels were held in place with magnetic strips.

Approximately 5-10 µl of sample was applied to the surface of the gel with a draftsman's lining pen. Between 50 and 60 samples were loaded on a 15 cm x 30 cm gel. Each run included at least two standards. Optimal electrophoretic conditions for individual loci are listed in the Appendix 2c.

After electrophoresis the gel was treated with 2 ml of the appropriate histochemical stain (Appendix 2c) for 1 min. The stock solutions used in histochemical staining are listed in Appendix 2d. Gels were blotted to remove excess staining solution, heat-sealed in plastic bags and allowed to incubate at 37°C or room temperature as appropriate. Staining reactions

were stopped with 5% formalin solution. The gels were scored, photocopied and kept in sealed plastic bags at room temperature for up to 6 months.

2.2.5 Double staining

On most gels it was possible to score two enzymes. The gels were stained for the first enzyme, scored and than stained for the second enzyme. The following combinations were used in the study of *L. sealii*: aGPD/GOT, PGD/G6PD and MPI/LDH2.

In the *G. maculatus* study only one combination lent itself to double staining: GPI/PGM.

2.3 Analytical Procedures

2.3.1 Genetic nomenclature

Patterns of enzyme variation that were consistent with the known subunit structure of the enzyme (Shaklee and Keenan, 1986) were used for discrimination of stocks. Names of enzymes and Enzyme Commission numbers follow the recommendations of the Nomenclature Committee of the American Fisheries Society (Shaklee *et al.*, 1990). For multilocus enzyme systems the form with the least anodal migration was designated "1" , the next "2" eg. GOT1 and GOT2. For each locus, alleles are indicated by Arabic numbers with the most anodally migrating allele designated "*1", and so on. For example the following convention was used to identify allele 2 at locus PEPA; PEPA*2. Multiple alleles in a genotype were separated by forward slashes in the following manner; PEPA*1/2.

There were no loci in the present study that exhibited cathodal migration.

The electrophoretic patterns and their isozymic interpretation have been previously described for all enzymes (Kornfield *et al.*, 1981, 1982) and Mendelian inheritance confirmed by breeding studies (King, 1983). The putative genotype data were tabulated as genotype and allele frequency distributions, for each species, in the form suitable for input into the statistical programs described below.

2.3.2 Genetic data analysis

Relationships among the collections were examined by the POPSEP and GENESTATS programs.Unrooted tree (network) is constructed from Nei's genetic distance data generated from allele frequency data in the GENESTATS program.

The G-test (Sokal and Rohlf, 1981) provides a simple, yet powerful test for distingiushing populations, and uses all the gene frequency data available. The program POPSEP (Centre for Marine Studies, UNSW), performs G-tests on all possible pairs of populations. In order to reduce the bias of very small sample sizes the data was then subjected to further analysis using Fisher's Exact Probability Test. Fisher's Exact Test is a nonparametric and distribution-free test; under the null hypothesis of independence, its distribution is known (Fisher and van Belle, 1993).

The genetic distance between pairs of populations was determined using the program "Neistat". This computes Nei's genetic distance D* (as modified by Hillis, 1984) from allele frequency distributions.

The computer program "Genestats" was used to perform the following calculations based on genotype data: allele frequency calulations, chi-square analysis on allele frequencies, test for departures from random mating, chi-square analysis on each genotypic class, chi-square analysis on observed and expected proportions of heterozygotes, F-statistics: Nei, F-statistics: Weir and Cockerham (Black and Krafsur, 1985 as modified by Woodburn 1989).

The genotype distributions of various loci in each species were examined for internal consistency with the Hardy-Weinberg distribution. The assumptions underlying the Hardy Weinberg Equilibrium are: there is random mating between genotypes; the population is large; there is no differential selection between genotypes; there is no differential immigration or emigration of genotypes; and there is no mutation.

Unless there is some disturbance of the frequency distibution, Hardy Weinberg Equilibrium will remain constant from one generation to the next. The interpretation is any distortions is the key to understanding population structure (Richardson *et al.*, 1986). The chi-squared test has been employed to assess the statistical significance of any divergence from expected frequency distributions. This allows a comparison of the "observed" and "expected" frequency of each genotypic class.

Heterogeneity between and within populations was investigated using Wright's (1943, 1951, 1965) F statistics. Using the three F-statistics, F_{IS}, F_{ST} and F_{IT}, the overall deviation from Hardy-Weinberg proportions was split into deviation caused by subpopulation differentiation from local Hardy-Weinberg proportions.

The BIOSYS-1 package conducted FSTAT analysis, a procedure for analysing genetic differentiation of populations by F-statistics (Wright, 1965; Nei, 1977). The mean values of F_{ST}, F_{IT} and F_{IS} were calculated across loci of all samples.

The G_{ST} analysis calculated Nei's gene diversity statistic G_{ST} (Nei, 1973). G_{ST} was estimated forn each locus by $(H_T-H_S)/(H_T$, where H_T represents that total heterozygosity and H_S the average (Hardy-Weinberg expected) sample heterozygosity. The proportion or magnitude of G_{ST} generated by sampling error, G_{STnull}, was estimated using a bootstrapping program, given the observed allele frequencies and sample sizes. For each test, 1000 randomisations of the data were run to provide the mean and standard deviation for G_{STnull}. The significance of the differentiation observed between samples is given by $P= n/1000$, where n is the number of randomisations that generated $G_{STnull} \geq G_{ST}$. Values of $P<0.05$ indicated significant differentiation between samples that could not be explained by sampling error.

2.4 Morphometric and Meristic Analysis

The degree of morphological divergence of *L. sealii* was investigated by the analysis of 27 morphometric and 5 meristic characters from 5 geographic regions throughout the specie range. The scheme of McDowall and Frankenberg (1981) was used to record measurements and counts. All measurements were made with vernier calipers, and recorded to the nearest millimeter. All meristic and morphometric counts were made with the aid of a low power

(Mag x10) dissecting microscope. All specimens were preserved in 10 % formalin until measurement.

Twenty female specimens were measured from each of the following Tasmanian rivers, Mersey River, Pieman River, Duck River, Derwent River, and the Lune River.

Males were not used for morphometric analysis as they were often caught post-spawning in a deteriorating physical condition. The measurements and meristic counts undertaken are given in Table 2.4 :

Table 2.4 Morphometric measurements and Meristic counts of *Lovettia sealii*

SL	Standard Length	PRE-D	Pre-Dorsal Length
LCF	Length to Caudal Fork	PRE-A	Pre-Anal Length
TL	Total Length	PRE-PEL	Pre-Pelvic Length
BDV	Body Depth at Vent	PEC-PEL	Pectoral to Pelvic Length
DCP	Depth of Caudal Peduncle	PEL-AN	Pelvic to Anal Length
LCP	Length of Caudal Peduncle	HL	Head Length
LDB	Length of Dorsal Base	D-A	Dorsal to Anal Length
MLD	Median Length to Dorsal Fin	SNL	Snout Length
LAB	Length of Anal Base	POHL	Post-orbital Head Length
MLA	Median Length to Anal Fin	IOW	Inter-orbital Width
PEC	Pectoral Fin Length	DE	Diameter of Eye
PEL	Pelvic Fin Length	LUJ	Length of Upper Jaw
LM	Length of Mandible	WG	Width of Gape
HDE	Head Depth to Eye		

Meristic Counts: Fin Rays
- Dorsal
- Caudal
- Anal
- Pectoral
- Pelvic

2.4.1 Canonical variate analysis

The standardized morphometric and meristic data were analysed by canonical variate analysis (CVA). The analysis was run on the Multigroup Discriminant analysis (MDA) program (version 2.0) available on the Biostat 11 package. Main features of the output included group means and standard deviations and within group and between group correlation matrices. These values were summarised in a classification matrix whereby each individual was assigned to the group it most closely resembles. Eigenvalues, the canonical correlations, coefficients, and values of each canonical variable evaluated from group means and the cumulative proportion of total dispersion absorbed by each canonical vector were listed. Finally, the values of the first, second and third canonical variables were given for each individual.

The cluster analysis was based on a matrix of Euclidean distances calculated from group means. The cluster dendrogram of the Euclidean distance matrix was formed by unpaired group average sorting (UPGMA) method. The degree of clustering of group average sorting is intermediate between the weakly clustering nearest neighbour and strongly clustering furthest neighbour sorting strategies (Johnson, 1979).

Chapter 3

3.0 Results: *Lovettia sealii*

3.1 Pilot study

Gene frequencies at 13 polymorphic loci are listed in Table 3.1. Screening of small samples of *L. sealii* indicated that loci *GOT2, LDH2, PEPA*, and *PGK* were worth investigating with larger samples of the species. The locus *PGK* was not incorporated in the main study as banding patterns were not readily interpreted when visualised under UV light. The loci *aGPD, PGD, G6PD* and *MPI* were included in the main survey. With the exception of *PEPA* all loci could be scored as pairs on a single gel due to double-staining techniques.

Table 3.1 Gene frequencies (%) of *L.sealii* in the Pilot Study

Locus	Allele	River				
		Derwent	Huon	Rubicon	Lune	Inglis
EST	2	94	100	100	100	100
	1	6				
GOT1	2	6				
	1	94	100	100	100	100
GOT2	2	44	56	32	56	44
	1	56	44	68	44	56
G6PD	2	94	94	94	94	94
	1	6	6	6	6	6
aGPD	3		6			
	2	100	94	94	81	69
	1			6	19	31
GPI2	2	94	100	100	94	100
	1	6			6	
LDH2	2	75	81	75	75	87
	1	25	19	25	25	13
MDH2	2			6		
	1	100	100	94	100	100
MPI	3	100	94	81	94	100
	2		6	13	6	
	1			6		
PEPA	2	43	31	100	75	87
	1	57	69		25	13
PGD	3				6	
	2	19	13	6	13	25
	1	81	87	94	81	75
PGK	2	44	62	56	38	50
	1	56	38	44	62	50
PGM	3		6		6	6
	2	94	94	100	94	94
	1	6				

3.2 Main Study *L.sealii*

3.2.1. Allele Frequencies

Of the 30 enzymes surveyed in *L. sealii* the following loci were selected for more detailed investigation: *aGPD, G6PD, PEPA, PGD, LDH2, GOT1, GOT2* and *MPI*. Allele frequencies for each of the polymorphic loci are shown in Table 3.2. Low level polymorphism (the frequency of the most common allele ≥ 0.95 in all samples) was observed at loci *MPI* and *GOT1*. All other loci displayed higher polymorphism (the frequency of the most common allele ≤ 0.9) in at least one sample. The highest level of polymorphism was observed at the *PEPA* and *GOT2* loci. There was also a marked variation in allele frequencies between samples at the *PEPA* locus. The frequency of allele *PEPA*4* varied from 0.874 to 0.970 in the north of the state while in the southeast, the range was 0.291 to 0.611. The frequency of this allele in the Pieman River on the west coast was 0.198.

3.2.2. Allele Frequency Analysis

The weighted mean allele frequencies are shown in Table 3.3. In addition a chi-square test for homogeneity of allele frequencies among samples is presented for each allele. A heterogeneity chi-square valaue has been calculated for each allele. The most heterogeneity across samples was seen at the *PEPA* locus. The most significant heterogeneity across samples was seen at alleles *PEPA*3* and *PEPA*4*.

The chi-square tests of specific alleles which showed significant heterogeneity are listed in Table 3.4. The contribution of each sample to the total chi-square is listed in Table 3.4. The samples that contributed the greatest amount to the resultant departure from equilibrium are underlined. The heterogeneity observed at *PEPA*3* and *PEPA*4* cannot be attributed to any particular sample as all samples appear to make an equal contribution. In contrast, the heterogeneity of *GPD*1* can be principally atributed to populations from the Tasmanain northcoast; Mersey 1/86, Rubicon/86 and Leven/86.

3.2.3 Analysis of Random Mating

The number of individuals observed at each genotypic class were calculated. To compensate for the small sample size of some classes a correction was performed when calculating the expected number of individuals by the Hardy-Weinberg rule. The total chi-square over all genotypes was analysed. The results of the pooled analysis at selected loci are shown in Appendix 3a. The observed and expected numbers of the following genotypic classes are presented: homozygotes for the most common allele, common/rare heterozygotes and rare homozygotes + other heterozygotes.

The Mersey/2/86 sample shows an excess of homozygotes (heterozygote deficiency) at the locus *6PGD* (observed 7, expected 12) this leads to a significant chi-square value $P = 0.003$ when the test for homogeneity was applied. Across the complete data set the only other samples to demonstrate heterozygote deficiencies were: Mersey/86 *PEPA* (obs. 17 vs exp.22), Leven/86 *6PGD* (obs. 13 vs exp. 23) and Huon/86 *LDH2* (obs. 22 vs exp. 29). The Leven/86 heterozygote deficiency at the *6PGD* locus was evidenced in the high F(IS) values (0.439) calculated for both alleles *6PGD*C* and *6PGD*D* (Appendix 3b).

In four samples heterozygote excess was noted: Mersey 5/86 *LDH2* (obs. 46 vs exp. 37), Inglis locus *LDH2* (obs. 40 vs exp. 31), Huon 2/85 *LDH2* (obs. 48 vs exp. 37) and Mersey 4/86 *GOT2* (obs. 59 vs exp. 48). The F(IS) value for Huon 2/85 at the *LDH2* locus was -0.298 to confirm the heterozygote excess observed for alleles *LDH2*B* and *LDH2*C*.

Table 3.2 Allele Frequencies in Subpopulations *L. sealii*

Loc.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.
GPD-1 (N)	98	100	100	100	96	95	99	89	41	96	99	97	100	100	100	100	36	68	100	100
1	0.133	0.075	0.025	0.055	0.115	0.032	0.030	0.000	0.000	0.010	0.000	0.000	0.050	0.035	0.000	0.110	0.000	0.000	0.000	0.010
2	0.867	0.925	0.975	0.945	0.885	0.968	0.965	1.000	1.000	0.990	1.000	1.000	0.945	0.960	1.000	0.890	1.000	1.000	1.000	0.980
3	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.000	0.000	0.000	0.010
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G6PD (N)	100	100	100	100	96	95	100	83	40	96	100	98	96	100	100	100	36	68	96	100
1	0.005	0.055	0.030	0.020	0.005	0.026	0.040	0.054	0.013	0.031	0.060	0.061	0.031	0.015	0.020	0.025	0.028	0.007	0.042	0.035
2	0.995	0.945	0.970	0.980	0.995	0.974	0.955	0.940	0.988	0.969	0.940	0.939	0.969	0.985	0.980	0.950	0.972	0.993	0.953	0.965
3	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.005	0.000
PEP-A (N)	96	98	100	100	96	95	100	81	41	96	100	98	100	100	99	100	35	68	98	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.005	0.010	0.000	0.000	0.000	0.000	0.012	0.012	0.005	0.000	0.036	0.060	0.015	0.030	0.000	0.000	0.000	0.005	0.005
3	0.042	0.061	0.055	0.050	0.042	0.116	0.550	0.370	0.463	0.604	0.685	0.668	0.050	0.035	0.051	0.030	0.600	0.544	0.531	0.650
4	0.958	0.929	0.910	0.900	0.958	0.884	0.445	0.611	0.524	0.375	0.315	0.291	0.875	0.890	0.874	0.970	0.400	0.449	0.464	0.345
5	0.000	0.005	0.025	0.050	0.000	0.000	0.005	0.006	0.000	0.016	0.000	0.005	0.015	0.060	0.045	0.000	0.000	0.007	0.000	0.000
6PGD (N)	102	100	100	100	96	95	100	89	40	95	100	98	100	88	99	95	29	68	96	100
1	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.956	0.935	0.890	0.860	0.870	0.800	0.870	0.876	0.988	0.895	0.930	0.872	0.915	0.818	0.854	0.858	0.845	0.882	0.880	0.860
4	0.044	0.065	0.105	0.140	0.130	0.184	0.125	0.124	0.013	0.105	0.065	0.122	0.085	0.182	0.131	0.142	0.155	0.103	0.115	0.130
5	0.000	0.000	0.005	0.000	0.000	0.011	0.005	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.015	0.000	0.000	0.015	0.005	0.010

TABLE 3.1 (CONT.)

LDH-2 (N)	101	80	92	100	96	79	100	89	34	95	100	96	98	99	99	100	35	66	64	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.168	0.256	0.212	0.245	0.240	0.266	0.220	0.202	0.191	0.237	0.255	0.260	0.265	0.242	0.242	0.155	0.171	0.152	0.164	0.180
3	0.822	0.744	0.788	0.750	0.760	0.734	0.780	0.798	0.809	0.763	0.735	0.740	0.735	0.758	0.758	0.845	0.829	0.841	0.836	0.820
4	0.010	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000

GOT-2 (N)	101	99	99	98	96	95	100	88	40	96	100	99	85	100	100	100	36	66	96	100
1	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.005	0.000	0.000	0.000	0.000	0.015	0.011	0.038	0.005	0.000	0.005	0.006	0.010	0.010	0.010	0.000	0.008	0.005	0.005
3	0.673	0.596	0.636	0.607	0.625	0.611	0.675	0.670	0.587	0.755	0.710	0.657	0.771	0.610	0.660	0.595	0.750	0.652	0.714	0.660
4	0.327	0.399	0.354	0.393	0.375	0.389	0.310	0.313	0.375	0.240	0.285	0.338	0.224	0.380	0.330	0.395	0.250	0.341	0.281	0.335
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MPI (N)	100	100	100	100	100	100	100	89	40	96	100	97	100	100	100	100	36	68	100	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.011	0.000	0.016	0.000	0.010	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
3	1.000	1.000	0.995	1.000	1.000	1.000	0.985	0.989	1.000	0.979	1.000	0.979	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000
4	0.000	0.000	0.005	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

GOT-1 (N)	100	100	100	100	100	100	99	100	40	101	100	99	100	100	100	100	36	68	100	100
1	0.995	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	0.985	1.000	1.000	1.000	1.000	1.000	0.956	1.000	0.985
2	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.044	0.000	0.015

TABLE 3.1 (CONT.)

Loc.	21.	22.	23.	24.	25.	26.	27.
GPD-1							
(N)	100	100	95	32	100	100	100
1	0.010	0.000	0.000	0.000	0.020	0.050	0.010
2	0.990	1.000	0.995	1.000	0.960	0.950	0.990
3	0.000	0.000	0.000	0.000	0.020	0.000	0.000
4	0.000	0.000	0.005	0.000	0.000	0.000	0.000
G6PD							
(N)	100	100	100	32	100	100	98
1	0.065	0.010	0.060	0.031	0.010	0.005	0.010
2	0.910	0.990	0.940	0.922	0.990	0.995	0.990
3	0.025	0.000	0.000	0.047	0.000	0.000	0.000
PEP-A							
(N)	100	99	88	32	96	100	100
1	0.000	0.010	0.000	0.000	0.000	0.000	0.000
2	0.005	0.020	0.000	0.031	0.010	0.000	0.000
3	0.630	0.424	0.511	0.672	0.792	0.075	0.210
4	0.355	0.545	0.489	0.297	0.198	0.915	0.730
5	0.010	0.000	0.000	0.000	0.000	0.010	0.060
6PGD							
(N)	100	100	100	32	100	100	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.860	0.970	0.900	0.875	0.845	0.885	0.875
4	0.140	0.030	0.085	0.125	0.155	0.110	0.115
5	0.000	0.000	0.015	0.000	0.000	0.005	0.010
LDH-2							
(N)	100	100	90	32	100	100	100
1	0.000	0.000	0.000	0.031	0.000	0.000	0.000
2	0.170	0.205	0.194	0.188	0.135	0.235	0.180
3	0.830	0.795	0.806	0.781	0.865	0.765	0.820
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GOT-2							
(N)	100	100	95	32	99	100	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.025	0.025	0.000	0.000	0.005	0.000	0.005
3	0.720	0.705	0.700	0.766	0.682	0.680	0.710
4	0.255	0.270	0.300	0.234	0.313	0.320	0.285
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 3.1 (CONT.)

Loc.	21	22	23	24	25	26	27
MPI							
(N)	100	100	100	32	100	100	100
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000	0.005	0.000
3	1.000	1.000	1.000	1.000	1.000	0.995	1.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GOT-1							
(N)	100	100	92	32	100	100	100
1	0.995	0.985	1.000	1.000	1.000	0.995	1.000
2	0.005	0.015	0.000	0.000	0.000	0.005	0.000

Weighted Mean

Table 3.3: Chi-square Analysis of Allele Frequencies in *L.sealii*

Locus	Weighted Mean	Chi-Square	Prob.	Heterogeneity Chi-square	d.f.	Prob.
GPD-1						
(N)	2441.		(26d.f.)			
1	0.031	241.65	0.0001			
2	0.967	224.35	0.0001			
3	0.002	53.51	0.0012			
4	0.000	24.70	0.5360			
				319.67	78	0.0001
G6PD						
(N)	2434.		(26d.f.)			
1	0.030	64.01	0.0000			
2	0.967	81.31	0.0001			
3	0.003	108.14	0.0001			
				172.59	52	0.0001
PEP-A						
(N)	2416.		(26d.f.)			
1	0.000	46.83	0.0074			
2	0.010	106.62	0.0001			
3	0.331	1604.67	0.0001			
4	0.646	1462.36	0.0001			
5	0.013	140.91	0.0001			
				1882.69	**	0.0001
6PGD						
(N)	2422.		(26d.f.)			
1	0.000	24.50	0.5474			
3	0.883	76.65	0.0001			
4	0.112	74.13	0.0000			
5	0.004	31.29	0.2177			
				130.40	78	0.0002
LDH-2						
(N)	2345.		(26d.f.)			
1	0.000	144.62	0.0001			
2	0.211	42.85	0.0200			
3	0.787	42.36	0.0225			
4	0.001	34.99	0.1119			
				222.32	78	0.0001
GOT-2						
(N)	2420.		(26d.f.)			
1	0.001	38.78	0.0511			
2	0.007	46.41	0.0082			
3	0.670	53.36	0.0012			
4	0.322	55.66	0.0006			
5	0.000	23.20	0.6213			
				163.37	**	0.0002
MPI						
(N)	2458.		(26d.f.)			
1	0.000	23.48	0.6056			
2	0.003	63.84	0.0001			
3	0.996	70.50	0.0000			
4	0.001	21.85	0.6971			
				109.24	78	0.0113
GOT-1						
(N)	2467.		(26d.f.)			
1	0.996	90.13	0.0001			
2	0.004	90.46	0.0001			
				90.46	26	0.0001
			Total:	3090.73	598	0.0001

TABLE 3.4 CONTRIBUTION OF L. SEALII POPULATIONS TO SUBSTRUCTURING

ALLELE	CHI SQUARE	PROB.	SAMPLE NO.									
			1	2	3	4	5	6	7	8	9	10
			11	12	13	14	15	16	17	18	19	20
			21	22	23	24	25	26	27			
GPD-1												
1	241.649	0.0001										
			0.277	0.053	0.001	0.016	0.183	0.000	0.000	0.024	0.011	0.011
			0.026	0.026	0.010	0.000	0.027	0.171	0.010	0.018	0.027	0.012
			0.012	0.027	0.025	0.009	0.003	0.010	0.012			
2	224.355	0.0001										
			0.269	0.049	0.002	0.013	0.177	0.000	0.000	0.027	0.013	0.014
			0.030	0.030	0.013	0.001	0.031	0.164	0.011	0.021	0.031	0.005
			0.015	0.031	0.021	0.010	0.001	0.008	0.015			
3	53.507	0.0012										
			0.007	0.007	0.007	0.007	0.007	0.007	0.021	0.006	0.003	0.007
			0.007	0.007	0.020	0.020	0.007	0.007	0.002	0.005	0.007	0.135
			0.007	0.007	0.007	0.002	0.670	0.007	0.007			
G6PD												
1	64.013	0.0000										
			0.067	0.067	0.000	0.011	0.063	0.001	0.011	0.052	0.013	0.000
			0.097	0.103	0.000	0.024	0.011	0.003	0.000	0.037	0.014	0.003
			0.132	0.043	0.097	0.000	0.043	0.067	0.041			
2	81.307	0.0001										
			0.061	0.036	0.001	0.013	0.058	0.004	0.011	0.046	0.013	0.000
			0.055	0.059	0.000	0.026	0.013	0.021	0.001	0.035	0.014	0.000
			0.246	0.041	0.055	0.049	0.041	0.061	0.040			
3	108.142	0.001										
			0.006	0.006	0.006	0.006	0.006	0.006	0.002	0.004	0.002	0.006
			0.006	0.006	0.006	0.006	0.006	0.266	0.002	0.004	0.002	0.006

			0.266 0.006 0.006 0.343 0.006 0.006 0.006
PEPA			
1	46.827	0.0074	0.002 0.002 0.002 0.002 0.002 0.002 0.002 0.001 0.001 0.002 0.002 0.002 0.002 0.002 0.002 0.002 0.001 0.001 0.002 0.002 0.002 0.959 0.002 0.001 0.002 0.002 0.002
PEPA			
2	106.620	0.0001	0.017 0.004 0.000 0.018 0.017 0.017 0.018 0.001 0.001 0.004 0.018 0.134 0.507 0.006 0.085 0.018 0.006 0.012 0.004 0.004 0.004 0.022 0.016 0.030 0.000 0.018 0.018
3	1604.665	0.0001	0.045 0.040 0.043 0.044 0.045 0.025 0.027 0.001 0.004 0.040 0.071 0.063 0.044 0.049 0.044 0.051 0.014 0.017 0.022 0.057 0.050 0.005 0.016 0.021 0.115 0.037 0.008
4	1462.365	0.0001	0.056 0.047 0.042 0.039 0.056 0.032 0.024 0.001 0.004 0.042 0.066 0.074 0.031 0.036 0.031 0.063 0.013 0.016 0.019 0.054 0.051 0.006 0.013 0.023 0.115 0.043 0.004
5	140.914	0.0001	0.018 0.007 0.016 0.151 0.018 0.018 0.007 0.004 0.008 0.001 0.019 0.007 0.000 0.243 0.115 0.019 0.007 0.002 0.018 0.019 0.001 0.019 0.016 0.006 0.018 0.001 0.007
6PGD			
3	76.654	0.0001	0.136 0.068 0.001 0.014 0.004 0.167 0.005 0.001 0.110 0.003 0.055 0.003 0.025 0.095 0.022 0.016 0.011 0.000 0.000 0.014 0.014 0.190 0.007 0.001 0.037 0.000 0.002
4	74.125	0.0000	0.128 0.061 0.001 0.021 0.008 0.133 0.004 0.003 0.108 0.001 0.061 0.003 0.020 0.115 0.010 0.023 0.014 0.002 0.000 0.008 0.021 0.183 0.020 0.001 0.049 0.000 0.000

LDH2			
1	144.624	0.0001	0.001 0.000 0.001 0.001 0.001 0.000 0.001 0.001 0.000 0.001 0.001 0.001 0.001 0.001 0.001 0.001 0.000 0.000 0.000 0.001 0.001 0.001 0.001 0.986 0.001 0.001 0.001
LDH2			
2	42.852	0.0200	0.052 0.046 0.000 0.032 0.022 0.066 0.002 0.002 0.004 0.018 0.054 0.065 0.081 0.027 0.027 0.088 0.015 0.066 0.040 0.027 0.047 0.001 0.007 0.005 0.162 0.016 0.027
3	42.362	0.0225	0.034 0.043 0.000 0.039 0.019 0.063 0.001 0.003 0.004 0.015 0.077 0.061 0.076 0.024 0.024 0.094 0.017 0.054 0.043 0.030 0.052 0.002 0.009 0.000 0.171 0.014 0.030
GOT2			
2	46.407	0.0082	0.030 0.002 0.029 0.029 0.028 0.028 0.043 0.012 0.240 0.002 0.030 0.002 0.000 0.006 0.006 0.006 0.011 0.000 0.002 0.002 0.210 0.210 0.028 0.009 0.002 0.030 0.002
3	53.357	0.0012	0.000 0.093 0.020 0.067 0.034 0.058 0.000 0.000 0.047 0.117 0.027 0.003 0.145 0.062 0.002 0.097 0.039 0.004 0.030 0.002 0.042 0.020 0.014 0.049 0.002 0.002 0.027
GOT2			
4	55.658	0.0006	0.000 0.097 0.016 0.081 0.045 0.071 0.002 0.001 0.019 0.107 0.022 0.004 0.135 0.056 0.001 0.088 0.031 0.004 0.026 0.003 0.074 0.044 0.008 0.040 0.001 0.000 0.022
MPI			
2	63.839	0.0001	0.010 0.010 0.010 0.010 0.010 0.010 0.050 0.061 0.004 0.156 0.010 0.053 0.010 0.010 0.496 0.010 0.003 0.007 0.010 0.010 0.010 0.010 0.010 0.003 0.010 0.004 0.010
3	70.503	0.0000	0.012 0.012 0.001 0.012 0.012 0.012 0.083 0.032 0.005 0.188

0.012 0.185 0.012 0.012 0.305 0.012 0.004 0.008 0.012 0.012
0.012 0.012 0.012 0.004 0.012 0.001 0.009

GOT1

1 90.127 0.0001

0.001 0.009 0.009 0.009 0.009 0.009 0.008 0.001 0.003 0.009
0.009 0.073 0.009 0.009 0.009 0.009 0.003 0.636 0.009 0.072
0.001 0.072 0.008 0.003 0.009 0.001 0.009

2 90.457 0.0001

0.001 0.009 0.009 0.009 0.009 0.009 0.008 0.001 0.003 0.009
0.009 0.073 0.009 0.009 0.009 0.009 0.003 0.635 0.009 0.072
0.001 0.072 0.008 0.003 0.009 0.001 0.009

3.2.4 F-statistics

The mean F-statistics over all loci are tabulated in Table (3.5a) and Table (3.5b). The mean level of genetic differentiation F_{ST} between populations of *L. sealii* was 0.097. This ranged from 0.004 at the *LDH-2* locus to a high level of differentiation contributed by the *PEP-A* locus (0.30). These values are very similar regardless of whether the Nei or Weir and Cockerham formulas are adopted. The mean heterozygote deficiency across all loci is given by the F_{IS} value 0.007 (Weir and Cockerham) and 0.002 (Nei). Positive values signify a deficiency of heterozygotes. The results are very heterogeneous, ranging from -0.04 at *LDH-2* to 0.09 at the *GPD-1* locus for the Weir and Cockerham data set. A very similar pattern emerges for the Nei calculation. The total fixation index, F_{IT}, for each locus ranges from -0.04 at LDH-2 to 0.32 at *PEP-A* (Table 3.5a and 3.5b).

3.2.5 G_{ST} analysis

3.2.5.1G_{ST} analysis of pooled data

Nei's gene diversity statistic (GST) values are listed in Table 3.5c. The values illustrate the extent of the genetic differentiation among samples. The Gst values range from 0.009 at the *LDH2* locus to 0.29 at the *PEPA* locus. Heterogeneity in the frequency of *PEPA* alleles contributed most to the Gst value, this being derived from alleles *PEPA*3* and *PEPA*4* (Table 3.4).

Table 3.5c G_{ST} Estimates for *L. sealii*

Locus	Nei G_{ST}	P value
GPD1	0.048	< 0.001
G6PD	0.016	< 0.001
PEPA	0.298	< 0.001
6PGD	0.017	< 0.001
LDH2	0.009	0.066
GOT2	0.013	< 0.001
MPI	0.015	0.004
GOT1	0.021	0.004

3.2.5.2 G-statistics between samples

G-tests were performed between all possible pairs of populations (Table 3.6).

Eleven of the samples collected were representative of northern Tasmanian rivers.
Potential temporal and spatial differences were examined in samples collected at the Mersey River over 3 successive spawning seasons. Samples M1-3/85 were collected during the course of a single fish run, from a single collection site, over a 1 - 2 hour period. Sample M5/85 was collected a few months later in the spawning season. Analysis of these samples indicated genetic differentiation within a run however, samples M2 , M3 and M5/85 were not statistically different from each other. The latter were also not significantly different to early season samples caught in 1986. M1/85 was significantly different to Mersey River *L. sealii* collected in 1985, 1986 and 1987.

M1 and M4/86 were collected early and late in the 1986 season respectively. A single sample M/87 respresents fish caught in the Mersey River in the third year of the study. Allele frequencies in the Mersey River in 1986 were stable and hence G-statistic values were low. They were however,

Table 3.5a *L. sealii*, Mean F-statistics Over All Loci: Nei

Locus(L)	F(IS(L))	F(ST(L))	F(IT(L))
aGPD	0.084	0.046	0.127
G6PD	-0.002	0.015	0.013
PEPA	0.025	0.303	0.320
PGD	0.022	0.015	0.037
LDH2	-0.054	0.009	-0.044
GOT2	0.007	0.011	0.018
MPI	0.035	0.013	0.047
GOT1	-0.023	0.018	-0.004
Mean	0.002	0.099	0.101

Table 3.5b *L. sealii*, Mean F-statistics Over All Loci: W & C

Locus(L)	F(IS(L))	F(ST(L))	F(IT(L))
aGPD	0.0900	0.0425	0.1287
G6PD	0.0035	0.0105	0.0139
PEPA	0.0304	0.3076	0.3287
PGD	0.0274	0.0103	0.0374
LDH2	-0.0479	0.0040	-0.0437
GOT2	0.0123	0.0061	0.0183
MPI	0.0402	0.0075	0.0474
GOT1	-0.0171	0.0137	-0.0031
Mean	0.0076	0.0973	0.1042
S.D.(X)	0.00616	0.03283	0.03570

Table 3.6: G-tests in *L. sealii*

OTU 1	VS	OTU 2	G STAT	D OF F	PROB.	
1/85M		2/85M	33.1886	16	.0070	*
		3/85M	48.3077	17	.0001	*
		85/R	10.5034	13	.6523	
		85/IL	62.5998	15	.0000	*
		85/LU	217.9307	19	.0000	*
		85/1H	259.7331	15	.0000	*
		85/2H	287.6938	20	.0000	*
		86/1M	53.6119	19	.0000	*
		86/2D	204.3733	17	.0000	*
		86/1H	238.7140	17	.0000	*
		87/CAT	253.5703	17	.0000	*
		87/D	177.4075	15	.0000	*
		87/NWBY	157.9288	16	.0000	*
		D/85	138.2578	19	.0000	*
		AL/85	186.1786	20	.0000	*
		86/DK	94.6764	18	.0000	*
		86/LEV	32.0491	15	.0063	*
		85/5M	20.0789	13	.0933	
		86/4M	66.0950	17	.0000	*
		87/PBC	148.5385	16	.0000	*
		87/PMN	310.7164	17	.0000	*
		86/D1	158.6281	16	.0000	*
		87/M	78.2628	15	.0000	*
		87/H	88.1806	16	.0000	*
2/85M		3/85M	24.2637	17	.1124	
		85/R	21.4543	15	.1229	
		85/IL	31.7477	17	.0162	
		85/LU	181.5478	18	.0000	*
		85/1H	223.9280	18	.0000	*
		85/2H	226.6093	19	.0000	*
		86/1M	30.8042	19	.0424	
		86/2D	155.5255	17	.0000	*
		86/1H	202.7780	17	.0000	*
		87/CAT	208.0025	16	.0000	*
		87/D	141.8072	17	.0000	*
		87/NWBY	137.6781	18	.0000	*
		D/85	97.1063	18	.0000	*
		AL/85	149.5209	20	.0000	*
		86/DK	52.4351	17	.0000	*
		86/LEV	33.6886	16	.0060	*
		85/5M	15.8511	16	.4634	
		86/4M	40.0841	16	.0008	*
		87/PBC	122.9931	16	.0000	*

3.6 cont.

	87/PMN	290.2663	17	.0000 *
	86/D1	142.4466	17	.0000 *
	87/M	76.3958	16	.0000 *
	87/H	70.8881	16	.0000 *
3/85M	85/R	35.1673	16	.0038 *
	85/IL	27.3781	17	.0527
	85/LU	162.2899	20	.0000 *
	85/1H	213.5712	18	.0000 *
	85/2H	211.4611	20	.0000 *
	86/1M	22.9791	21	.3451
	86/2D	144.3260	18	.0000 *
	86/1H	187.0258	18	.0000 *
	87/CAT	189.6584	18	.0000 *
	87/D	127.5967	17	.0000 *
	87/NWBY	124.4904	19	.0000 *
	D/85	76.4930	19	.0000 *
	AL/85	142.6883	21	.0000 *
	86/DK	32.2420	18	.0206
	86/LEV	44.9856	18	.0004 *
	85/5M	22.9330	17	.1514
	86/4M	22.3036	18	.2188
	87/PBC	119.3058	18	.0000 *
	87/PMN	270.7990	19	.0000 *
	86/D1	123.3035	18	.0000 *
	87/M	57.9874	16	.0000 *
	87/H	37.5282	17	.0029 *
85/R	85/IL	35.9316	14	.0011 *
	85/LU	206.6732	18	.0000 *
	85/1H	254.3972	15	.0000 *
	85/2H	266.9617	19	.0000 *
	86/1M	39.7168	19	.0036 *
	86/2D	193.1322	16	.0000 *
	86/1H	225.3971	16	.0000 *
	87/CAT	242.3036	16	.0000 *
	87/D	168.7826	14	.0000 *
	87/NWBY	152.9763	16	.0000 *
	D/85	124.7929	18	.0000 *
	AL/85	171.2546	19	.0000 *
	86/DK	77.1400	17	.0000 *
	86/LEV	20.8028	14	.1068
	85/5M	8.2347	13	.8280
	86/4M	42.2799	16	.0004 *
	87/PBC	144.1117	15	.0000 *
	87/PMN	300.6737	16	.0000 *

3.6 cont.

	86/D1	151.5296	16	.0000 *
	87/M	58.4419	14	.0000 *
	87/H	77.8185	15	.0000 *
85/IL	85/LU	144.9396	20	.0000 *
	85/1H	180.3844	16	.0000 *
	85/2H	182.0999	20	.0000 *
	86/1M	42.7251	21	.0034 *
	86/2D	112.5484	17	.0000 *
	86/1H	149.7707	17	.0000 *
	87/CAT	167.8100	18	.0000 *
	87/D	101.3064	15	.0000 *
	87/NWBY	108.9275	18	.0000 *
	D/85	72.3278	20	.0000 *
	AL/85	110.9155	20	.0000 *
	86/DK	55.3580	18	.0000 *
	86/LEV	45.7352	16	.0001 *
	85/5M	22.2239	15	.1021
	86/4M	41.8366	18	.0012 *
	87/PBC	109.1101	17	.0000 *
	87/PMN	229.7053	18	.0000 *
	86/D1	99.6162	17	.0000 *
	87/M	53.5338	15	.0000 *
	87/H	45.3999	16	.0001 *
85/LU	85/1H	28.7015	21	.1214
	85/2H	22.4126	20	.3185
	86/1M	177.6131	21	.0000 *
	86/2D	34.0149	19	.0183
	86/1H	23.1163	20	.2831
	87/CAT	22.4751	18	.2116
	87/D	30.8068	20	.0578
	87/NWBY	23.8775	20	.2478
	D/85	33.2646	19	.0224
	AL/85	18.5093	21	.6166
	86/DK	172.7765	19	.0000 *
	86/LEV	231.5546	18	.0000 *
	85/5M	189.1753	19	.0000 *
	86/4M	192.4346	19	.0000 *
	87/PBC	49.1976	19	.0002 *
	87/PMN	46.4284	20	.0007 *
	86/D1	24.4347	20	.2239
	87/M	201.4332	19	.0000 *
	87/H	81.3777	19	.0000 *
85/1H	85/2H	27.7121	20	.1164

3.6 cont.

	86/1M	234.6853	21	.0000 *
	86/2D	37.7107	17	.0027 *
	86/1H	23.7593	18	.1631
	87/CAT	38.4642	19	.0052 *
	87/D	22.7098	15	.0905
	87/NWBY	26.8962	17	.0596
	D/85	60.1319	20	.0000 *
	AL/85	37.6434	21	.0142
	86/DK	232.3542	18	.0000 *
	86/LEV	287.7250	17	.0000 *
	85/5M	227.3588	15	.0000 *
	86/4M	263.8918	19	.0000 *
	87/PBC	56.2196	17	.0000 *
	87/PMN	54.7134	19	.0000 *
	86/D1	25.9767	16	.0544
	87/M	256.4740	16	.0000 *
	87/H	127.1615	17	.0000 *
85/2H	86/1M	217.8500	23	.0000 *
	86/2D	44.0185	19	.0009 *
	86/1H	25.4591	20	.1844
	87/CAT	36.1371	20	.0148
	87/D	39.8096	19	.0035 *
	87/NWBY	23.3679	21	.3247
	D/85	49.6056	20	.0003 *
	AL/85	35.3487	21	.0258
	86/DK	208.7508	18	.0000 *
	86/LEV	289.9506	20	.0000 *
	85/5M	241.6993	20	.0000 *
	86/4M	243.0864	20	.0000 *
	87/PBC	60.5708	19	.0000 *
	87/PMN	49.8022	21	.0004 *
	86/D1	33.4197	19	.0215
	87/M	259.9228	19	.0000 *
	87/H	131.9787	19	.0000 *
86/1M	86/2D	173.4272	20	.0000 *
	86/1H	204.6776	20	.0000 *
	87/CAT	206.7070	19	.0000 *
	87/D	158.8522	21	.0000 *
	87/NWBY	131.7510	20	.0000 *
	D/85	94.5036	21	.0000 *
	AL/85	155.7218	22	.0000 *
	86/DK	42.5631	21	.0036 *
	86/LEV	49.4282	19	.0002 *
	85/5M	28.5936	19	.0727

3.6 cont.

	86/4M	18.1867	19	.5100
	87/PBC	131.9397	19	.0000 *
	87/PMN	280.3694	20	.0000 *
	86/D1	139.7991	20	.0000 *
	87/M	64.8405	20	.0000 *
	87/H	64.3909	20	.0000 *
86/2D	86/1H	27.9397	17	.0456
	87/CAT	32.3888	17	.0135
	87/D	18.7863	16	.2799
	87/NWBY	25.1162	17	.0921
	D/85	32.6756	18	.0183
	AL/85	31.4544	20	.0495
	86/DK	152.6819	17	.0000 *
	86/LEV	201.7180	16	.0000 *
	85/5M	171.0601	17	.0000 *
	86/4M	188.0257	18	.0000 *
	87/PBC	43.0501	16	.0003 *
	87/PMN	65.7365	18	.0000 *
	86/D1	30.4151	17	.0235
	87/M	194.2883	17	.0000 *
	87/H	79.7520	17	.0000 *
86/1H	87/CAT	23.3523	18	.1774
	87/D	23.3631	17	.1378
	87/NWBY	23.4679	19	.2174
	D/85	47.0475	20	.0006 *
	AL/85	16.7191	20	.6711
	86/DK	213.0207	18	.0000 *
	86/LEV	241.2556	17	.0000 *
	85/5M	205.6398	17	.0000 *
	86/4M	218.3673	17	.0000 *
	87/PBC	49.1255	17	.0001 *
	87/PMN	20.1284	17	.2677
	86/D1	17.8133	18	.4680
	87/M	221.5097	17	.0000 *
	87/H	101.7345	17	.0000 *
87/CAT	87/D	38.2798	18	.0036 *
	87/NWBY	15.9498	18	.5960
	D/85	39.8202	18	.0022 *
	AL/85	21.0494	20	.3942
	86/DK	216.6986	18	.0000 *
	86/LEV	234.7682	16	.0000 *
	85/5M	220.7382	17	.0000 *
	86/4M	220.0487	17	.0000 *

3.6 cont.

	87/PBC	67.7047	17	.0000 *
	87/PMN	43.3342	18	.0007 *
	86/D1	33.1914	18	.0158
	87/M	235.9382	17	.0000 *
	87/H	104.6323	17	.0000 *
87/D	87/NWBY	31.3166	17	.0183
	D/85	27.8811	19	.0857
	AL/85	26.6682	20	.1449
	86/DK	147.3821	17	.0000 *
	86/LEV	188.7717	16	.0000 *
	85/5M	145.5858	15	.0000 *
	86/4M	175.1807	18	.0000 *
	87/PBC	35.3053	16	.0036 *
	87/PMN	71.3019	18	.0000 *
	86/D1	18.8645	16	.2758
	87/M	172.0487	15	.0000 *
	87/H	65.2388	16	.0000 *
87/NWBY	D/85	34.5249	19	.0159
	AL/85	29.8385	22	.1224
	86/DK	134.2136	19	.0000 *
	86/LEV	155.1040	17	.0000 *
	85/5M	141.8286	16	.0000 *
	86/4M	147.8001	19	.0000 *
	87/PBC	42.2712	17	.0006 *
	87/PMN	28.0331	19	.0828
	86/D1	29.6191	18	.0413
	87/M	148.1367	18	.0000 *
	87/H	76.1497	19	.0000 *
D/85	AL/85	29.4373	21	.1039
	86/DK	89.0477	18	.0000 *
	86/LEV	126.7646	18	.0000 *
	85/5M	102.0004	19	.0000 *
	86/4M	105.8236	19	.0000 *
	87/PBC	32.4631	18	.0194
	87/PMN	96.0189	20	.0000 *
	86/D1	29.0093	19	.0658
	87/M	130.9344	19	.0000 *
	87/H	41.1824	19	.0023 *
AL/85	86/DK	168.1429	20	.0000 *
	86/LEV	180.6444	19	.0000 *
	85/5M	155.2663	20	.0000 *
	86/4M	167.5711	20	.0000 *

3.6 cont.

	87/PBC	48.5569	21	.0006 *
	87/PMN	50.6539	21	.0003 *
	86/D1	19.6145	20	.4823
	87/M	173.3117	19	.0000 *
	87/H	70.7882	19	.0000 *
86/DK	86/LEV	91.2117	18	.0000 *
	85/5M	61.2792	18	.0000 *
	86/4M	37.6007	18	.0044 *
	87/PBC	125.5230	17	.0000 *
	87/PMN	302.4678	19	.0000 *
	86/D1	135.6321	17	.0000 *
	87/M	77.5525	17	.0000 *
	87/H	55.4734	17	.0000 *
86/LEV	85/5M	23.6221	15	.0718
	86/4M	44.9395	17	.0002 *
	87/PBC	173.6139	16	.0000 *
	87/PMN	317.7266	17	.0000 *
	86/D1	170.0709	17	.0000 *
	87/M	71.6309	16	.0000 *
	87/H	91.6914	16	.0000 *
85/5M	86/4M	33.8818	17	.0087 *
	87/PBC	127.5715	16	.0000 *
	87/PMN	288.8694	17	.0000 *
	86/D1	134.3036	16	.0000 *
	87/M	57.1179	15	.0000 *
	87/H	64.0035	16	.0000 *
86/4M	87/PBC	150.8011	17	.0000 *
	87/PMN	292.4796	17	.0000 *
	86/D1	147.0077	18	.0000 *
	87/M	53.7695	17	.0000 *
	87/H	51.8562	17	.0000 *
87/PBC	87/PMN	93.6500	17	.0000 *
	86/D1	24.9643	17	.0955
	87/M	164.5783	17	.0000 *
	87/H	65.4405	17	.0000 *
87/PMN	86/D1	47.2736	19	.0003 *
	87/M	302.1706	18	.0000 *
	87/H	165.4376	18	.0000 *
86/D1	87/M	141.9353	16	.0000 *

3.6 cont.

	87/H	50.4056	16	.0000 *
87/M	87/H	71.8724	15	.0000 *

significantly different to *L. sealii* caught the following season. A variable pattern emerges when G-tests were performed between 1985 (M2, M3 and M5) and 1986 Mersey River *L. sealii*: M2, 3 and 5 were not significantly different to early run (1986) *L. sealii* but, M2 and M5 could be separated from schools capteured later in the 1986 season.

G-statistic data reveal population sub-structuring in spawning runs both within and between successive spawning seasons in the Mersey River.

G-statistic relationships between north coast rivers in 1985 are variable. With the exception of M3/85 all other Mersey River samples are not significantly different to fish collected in the nearby Rubicon River in the same season. The Inglis River fish captured to the west of the Mersey and Rubicon are genetically distinct from the Mersey (M1/85) and Rubicon (R/85) river samples.

High levels of genetic variation are apparent in 1986 north coast fish. Samples from the far west (Duck River) to the central coast (Mersey and Leven Rivers) are genetically distinct on the basis of G-statistic data.

The sole 1987 sample from the Mersey River does not bear genetic resemblance to any other northern sample collected during the study.

Fifteen samples are representative of southern and southeastern-Tasmania. These were captured from the Derwent River (fig.2.1, site 8)to the Catamaran (fig. 2.1, site 12) in the far south of the state.

Multiple samples were collected from the Derwent and Huon Rivers in order to establish the temporal and spatial stability of gene frequencies of *L. sealii*. The relationship between samples captured within the same year are have been investigated by applying G-tests between populations. The results are summarised in Table 3.7.

Table 3.7 Summary of G-tests between samples of *L. sealii* in Southern Tasmanian Rivers, within 3 successive spawning seasons (1985-1987).

1985	2	3	4	5	6
1. Derwent /85		*	*		
2. Huon 1/85					*
3. Huon 2/85					*
4. Allen's /85					
5. Lune /85					
6. Parson's Bay /85					
1986					
1. Derwent 1/86					
2. Derwent 2/86					
3. Huon /86					
4. Catamaran /86					
No significant difference between any samples collected in 1986.					
1987	2	3	4	5	
1. Derwent/87		*		*	
2. Huon /87			*		
3. Catamaran /87			*		
4. NorthWest Bay /87				*	
5. Parson's Bay /87					

* ($P < 0.05$) significant G-test

The G-statistic values indicate that there is no significant difference between Derwent River specimens caught in 1985, 1986 or 1987. Multiple samples captured in 1986 did not differ from each other statistically.

A similar result was observed in the Huon River; samples collected over 3 successive seasons were not significantly different and duplicate samples within the 1985 season were also closely related.

A very different result is seen when comparing G-statistic values between the Derwent and Huon Rivers. Significant differences were observed between the 1985 Derwent sample and all other Huon representatives. A more complex pattern emerges with 1986 Derwent representatives. The early run Derwent specimens (D1/86) do not have G-statistic values which differ significantly from any other Huon fish. However, the late-run fish (D2/86) although not significantly different to 1986 or 1987 Huon samples, are distinct from 1985 specimens. In summary, the allele frequency data from *L. sealii* caught in the Derwent or Huon Rivers in 1987, 1986, or early 1985 specimens were not statistically different on the basis of G-statistic data. They were however, significantly different from late-run 1986 Derwent specimens. Therefore *L.sealii* caught in the Huon and Derwent Rivers in a single spawning season may represent unique genetic pools.

L. sealii caught in the NorthWest Bay River, which lies between the Derwent and Huon Rivers are not genetically distinct from representatives of either river nor any other sample of Southern Tasmanian, regardless of the year of capture. This is also true for the Lune River (South-east coast) and Allen's Creek (Tasman Peninsula) fish.

Catamaran River fish were not genetically distinct from their nearest neighbours but, G-statistic data revealed statistically significant differences between the Catamaran and Derwent River *L. sealii* captured in 1987.

Parson's Bay Creek representatives appeared to share no genetic links with other southern river fish caught in 1987. However, this sample was not significantly different to specimens captured in Parson's Bay Creek in 1985. There is no consistent pattern in the relationship between *L. sealii* caught in this creek and other south Tasmanian fish.

A review of 1985 samples of *L. sealii* in southern Tasmania would have led to the conclusion that there were 3 genetic pools: Derwent, Parson's Bay Creek and a third pool to include all other southern sites. In 1986 the more limited sampling would have concluded that all southern fish were all derived from a single genetic pool. In 1987, the 1985 scenario is repeated.

On the basis of G-statistic data the single sample of *L. sealii* from the west coast of Tasmania caught in the Pieman River in 1987 is significantly different from all other study samples except that of the NorthWest Bay River 1987 and early run Huon River fish captured in 1986.

In summary, of the 351 G-tests performed between populations 249 were found to be significant (Table 3.8). Northern Tasmanian fish are significantly different from those found in the south and south-east of the state. The west coast (Pieman River) appears to constitute a separate genetic pool. Temporal genetic stability is evident in the south but not the north of the state. Complex population sub-structuring was observed in both the northern and southern rivers.

Table 3.8 Summary of the results of G-tests performed between all *L. sealii* samples.

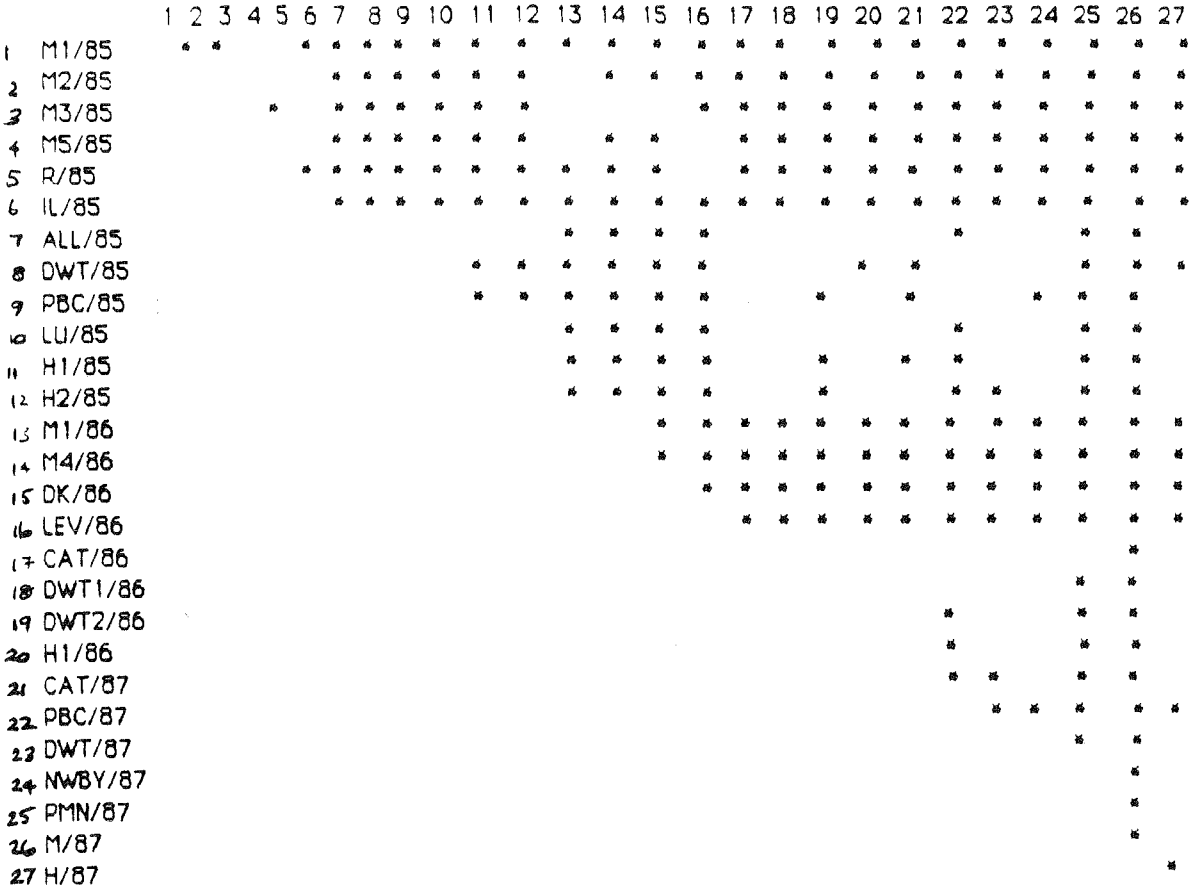


Table 3.11: Nei's Genetic Identity (I) and Distance (D)

I ↓ D →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 1/85M		0.0033	0.0019	0.0029	0.0015	0.0064	0.0518	0.0205	0.0355	0.0677	0.0846	0.0884	0.0036	0.0045	0.0038	0.0025	0.0640	0.0510	0.0479	0.0767	0.0749	0.0300	0.0430	0.0875	0.1109	0.0033	0.0754
2 2/85M	0.9967		0.0023	0.0003	0.0007	0.0023	0.0497	0.0195	0.0319	0.0674	0.0816	0.0824	0.0020	0.0019	0.0019	0.0027	0.0653	0.0479	0.0483	0.0744	0.0753	0.0267	0.0421	0.0883	0.1106	0.0034	0.0749
3 3/85M	0.9981	0.9977		0.0015	0.0017	0.0026	0.0471	0.0164	0.0325	0.0623	0.0788	0.0815	0.0010	0.0016	0.0010	0.0027	0.0591	0.0464	0.0435	0.0715	0.0695	0.0269	0.0387	0.0818	0.1056	0.0008	0.0705
4 85/5M	0.9971	0.9997	0.9985		0.0005	0.0015	0.0502	0.0194	0.0330	0.0679	0.0830	0.0838	0.0014	0.0008	0.0014	0.0019	0.0653	0.0483	0.0484	0.0750	0.0756	0.0276	0.0427	0.0885	0.1107	0.0021	0.0752
5 85/R	0.9985	0.9993	0.9983	0.9995		0.0026	0.0514	0.0205	0.0346	0.0687	0.0844	0.0859	0.0016	0.0016	0.0020	0.0017	0.0661	0.0500	0.0494	0.0766	0.0767	0.0291	0.0438	0.0895	0.1121	0.0021	0.0764
6 85/IL	0.9936	0.9977	0.9974	0.9985	0.9974		0.0424	0.0150	0.0291	0.0592	0.0739	0.0735	0.0019	0.0008	0.0025	0.0035	0.0568	0.0412	0.0413	0.0657	0.0661	0.0239	0.0364	0.0785	0.0995	0.0016	0.0662
7 AL/85	0.9495	0.9515	0.9540	0.9510	0.9499	0.9585		0.0087	0.0047	0.0023	0.0054	0.0061	0.0487	0.0515	0.0488	0.0539	0.0020	0.0008	0.0009	0.0033	0.0034	0.0058	0.0010	0.0068	0.0147	0.0458	0.0031
8 D/85	0.9797	0.9807	0.9838	0.9808	0.9797	0.9851	0.9914		0.0051	0.0165	0.0260	0.0277	0.0181	0.0201	0.0180	0.0220	0.0147	0.0089	0.0071	0.0213	0.0200	0.0033	0.0052	0.0276	0.0433	0.0162	0.0207
9 85/PBC	0.9651	0.9686	0.9681	0.9675	0.9660	0.9713	0.9953	0.9949		0.0118	0.0160	0.0164	0.0347	0.0361	0.0334	0.0369	0.0107	0.0029	0.0050	0.0119	0.0141	0.0004	0.0033	0.0200	0.0298	0.0335	0.0125
10 85/LU	0.9346	0.9349	0.9396	0.9344	0.9336	0.9426	0.9977	0.9836	0.9883		0.0020	0.0042	0.0641	0.0694	0.0638	0.0732	0.0008	0.0046	0.0030	0.0030	0.0013	0.0135	0.0040	0.0021	0.0102	0.0609	0.0016
11 85/1H	0.9189	0.9217	0.9242	0.9204	0.9191	0.9288	0.9946	0.9744	0.9842	0.9980		0.0012	0.0804	0.0856	0.0797	0.0896	0.0037	0.0071	0.0076	0.0022	0.0024	0.0189	0.0086	0.0019	0.0061	0.0779	0.0017
12 85/2H	0.9154	0.9209	0.9217	0.9196	0.9177	0.9292	0.9939	0.9727	0.9837	0.9958	0.9988		0.0819	0.0856	0.0818	0.0902	0.0060	0.0071	0.0095	0.0020	0.0040	0.0195	0.0106	0.0037	0.0057	0.0798	0.0028
13 86/1M	0.9964	0.9980	0.9990	0.9986	0.9984	0.9981	0.9524	0.9820	0.9659	0.9380	0.9228	0.9214		0.0013	0.0005	0.0040	0.0623	0.0487	0.0466	0.0743	0.0726	0.0289	0.0412	0.0843	0.1093	0.0008	0.0735
14 86/4M	0.9955	0.9981	0.9984	0.9992	0.9984	0.9992	0.9498	0.9801	0.9646	0.9330	0.9179	0.9179	0.9987		0.0019	0.0019	0.0664	0.0501	0.0495	0.0765	0.0765	0.0303	0.0444	0.0897	0.1117	0.0012	0.0768
15 86/DK	0.9962	0.9981	0.9990	0.9986	0.9980	0.9975	0.9524	0.9822	0.9671	0.9382	0.9234	0.9215	0.9995	0.9981		0.0050	0.0622	0.0482	0.0464	0.0742	0.0728	0.0279	0.0408	0.0844	0.1097	0.0016	0.0733
16 86/LEV	0.9975	0.9973	0.9973	0.9981	0.9983	0.9965	0.9475	0.9782	0.9638	0.9295	0.9143	0.9137	0.9960	0.9981	0.9951		0.0684	0.0519	0.0509	0.0782	0.0785	0.0313	0.0462	0.0927	0.1126	0.0032	0.0786
17 86/CAT	0.9380	0.9368	0.9426	0.9368	0.9360	0.9448	0.9980	0.9854	0.9894	0.9992	0.9963	0.9940	0.9396	0.9358	0.9397	0.9339		0.0037	0.0015	0.0025	0.0008	0.0122	0.0030	0.0025	0.0096	0.0581	0.0012
18 86/D1	0.9503	0.9533	0.9546	0.9528	0.9512	0.9596	0.9992	0.9912	0.9971	0.9954	0.9930	0.9930	0.9525	0.9511	0.9529	0.9495	0.9963		0.0016	0.0035	0.0053	0.0040	0.0016	0.0092	0.0155	0.0458	0.0040
19 86/2D	0.9532	0.9529	0.9574	0.9528	0.9518	0.9595	0.9991	0.9929	0.9950	0.9970	0.9924	0.9905	0.9545	0.9517	0.9547	0.9504	0.9985	0.9984		0.0047	0.0036	0.0056	0.0004	0.0074	0.0165	0.0430	0.0039
20 86/1H	0.9262	0.9283	0.9310	0.9278	0.9262	0.9364	0.9967	0.9789	0.9882	0.9970	0.9978	0.9980	0.9284	0.9263	0.9285	0.9248	0.9976	0.9965	0.9953		0.0014	0.0145	0.0063	0.0027	0.0044	0.0704	0.0004
21 87/CAT	0.9278	0.9274	0.9328	0.9272	0.9262	0.9360	0.9966	0.9802	0.9860	0.9987	0.9976	0.9960	0.9300	0.9263	0.9298	0.9245	0.9992	0.9947	0.9964	0.9986		0.0165	0.0056	0.0012	0.0059	0.0683	0.0007
22 87/PBC	0.9704	0.9736	0.9735	0.9728	0.9714	0.9764	0.9943	0.9967	0.9996	0.9865	0.9813	0.9807	0.9715	0.9702	0.9725	0.9692	0.9879	0.9960	0.9944	0.9856	0.9836		0.0037	0.0228	0.0340	0.0278	0.0150
23 87/D	0.9579	0.9587	0.9621	0.9582	0.9572	0.9642	0.9990	0.9948	0.9967	0.9961	0.9915	0.9895	0.9596	0.9565	0.9600	0.9548	0.9970	0.9984	0.9996	0.9937	0.9944	0.9963		0.0098	0.0203	0.0385	0.0057
24 87/NWBY	0.9162	0.9155	0.9215	0.9153	0.9144	0.9245	0.9932	0.9728	0.9802	0.9979	0.9981	0.9964	0.9191	0.9142	0.9191	0.9115	0.9975	0.9909	0.9926	0.9974	0.9988	0.9775	0.9902		0.0042	0.0803	0.0014
25 87/PMN	0.8950	0.8953	0.8998	0.8952	0.8939	0.9053	0.9854	0.9576	0.9707	0.9898	0.9939	0.9944	0.8965	0.8943	0.8961	0.8935	0.9904	0.9846	0.9837	0.9956	0.9941	0.9666	0.9799	0.9958		0.1039	0.0048
26 87/M	0.9967	0.9966	0.9992	0.9979	0.9979	0.9984	0.9552	0.9840	0.9670	0.9409	0.9250	0.9233	0.9992	0.9988	0.9984	0.9968	0.9436	0.9552	0.9579	0.9320	0.9340	0.9726	0.9622	0.9229	0.9013		0.0694
27 87/H	0.9274	0.9278	0.9319	0.9275	0.9265	0.9360	0.9969	0.9795	0.9876	0.9984	0.9983	0.9972	0.9292	0.9261	0.9293	0.9244	0.9988	0.9960	0.9961	0.9996	0.9993	0.9852	0.9943	0.9986	0.9952	0.9330	

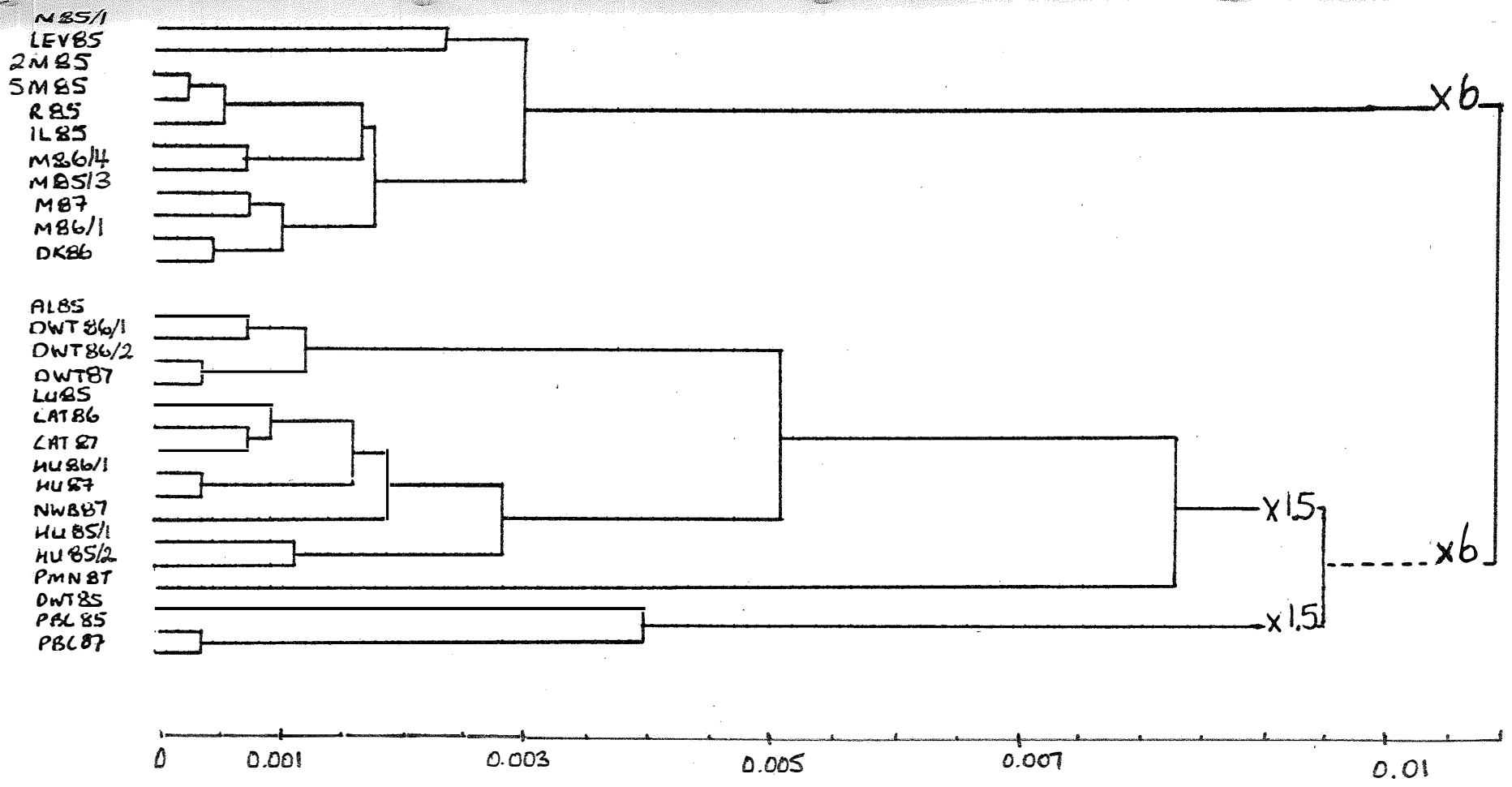


Figure 3.1: Unrooted relationship matrix for *L. sealii*

Genetic distance UPGMA

3.2.6 Nei's Genetic Distance (D) and Identity (I)

Nei's Distance (D) and Identity (I) values are shown in table (3.11). There is a low level of differentiation between populations. The genetic distance values range from 0.0003 to 0.1126. The genetic identity from 0.8939 to 0.997. The highest values were found with comparisons between the Pieman River on the west coast and the Leven River in the north which can principally be attributed to differences at the *PEP-D* locus.

An unrooted tree was produced using Nei D data for all *L. sealii* samples (Fig. 3.1). The figure illustrates a clear separation between north and south of the species range. Parson's Bay Creek on the Tasman Peninsula and the sample collected from the Derwent River in 1985 form another group within the southern split and finally the Pieman River of the west coast is separated from the general southern grouping.

3.2.7 Morphometric and Meristic Data Analysis

3.2.7.1 Merisitics

A summary of the fin ray counts in *L. sealii* samples is shown in Table 3.12.

Table 3.12 Summary of Fin Ray Counts (Mean ± SD) in L. sealii

Site	Dorsal	Caudal	Anal	Pectoral	Pelvic
Lune	7.80 ± 0.70	16.00 ± 0.00	17.05 ± 0.94	11.30 ± 0.66	7.05 ± 0.39
Duck	8.00 ± 0.65	16.00 ± 0.00	17.00 ± 1.03	11.05 ± 0.60	7.05 ± 0.39
Pieman	8.25 ± 0.55	16.00 ± 0.00	17.30 ± 0.92	11.60 ± 0.60	7.05 ± 0.22
Mersey	8.35 ± 0.49	16.00 ± 0.00	16.90 ± 0.79	11.30 ± 0.80	7.35 ± 0.49
Derwent	8.25 ± 0.44	15.95 ± 0.22	17.10 ± 1.07	10.75 ± 0.79	7.10 ± 0.31

3.2.7.2 Canonical variance analysis

The Euclidean distances shown in Table 3.13 show distinct separation between all rivers. The correct classification of samples to their river ranged from 85-100%. In scatter plots of group centroids all groups are separated using 1st and 2nd canonical axes (fig. 3.2) or 1st and 3rd canonical axes plots (fig 3.3).

The first canonical variant accounted for 46.97% of the variation between samples. The second and third canonical variants accounted for 30.66 and 11.39% of the sample variation respectively. Therefore together they account for 89.01% of sample variance. The descriptors which contributed most to these variants were diameter of eye, head depth to eye and inter-orbital width. The normalised canonical vectors are shown in Table 3.15.

Table 3.13 Euclidean distances between groups

	Derwent	Lune	Mersey	Duck	Pieman
Derwent	-	3.141	4.978	3.350	5.425
Lune		-	3.845	3.175	5.922
Mersey			-	4.629	6.942
Duck				-	5.141
Pieman					-

Table 3.14 Percentage of idividuals classifed into their correct river sample

	Derwent	Lune	Mersey	Duck	Pieman
Derwent	100	0	0	0	0
Lune	5	80	5	5	5
Mersey	0	0	95	5	0
Duck	0	0	5	95	0
Pieman	5	5	0	0	90

Table 3.15 Normalised cannonical vectors for raw data.

Variable No.	1	2	3
1	.308	-.208	.022
2	.022	.155	-.038
3	.031	.294	.008
4	-.037	.003	-.150
5	.084	-.089	-.058
6	-.037	.026	.073
7	-.004	.117	-.250
8	-.069	.046	.052
9	.109	.077	.129
10	-.028	.023	-.003
11	.083	-.116	-.072
12	0.0	-.011	-.014
13	.001	-.006	.114
14	-.038	-.014	.031
15	-.152	-.028	-.029
16	.050	.095	.123
17	.111	.172	-.064
18	-.151	-.050	-.036
19	.471	.466	.198
20	-.699	-.298	.740
21	-.217	.224	.252
22	.082	-.396	-.206
23	-.197	.068	-.244
24	-.060	.491	.304

Fig. 3.2: Group Centroids on two discriminat functions

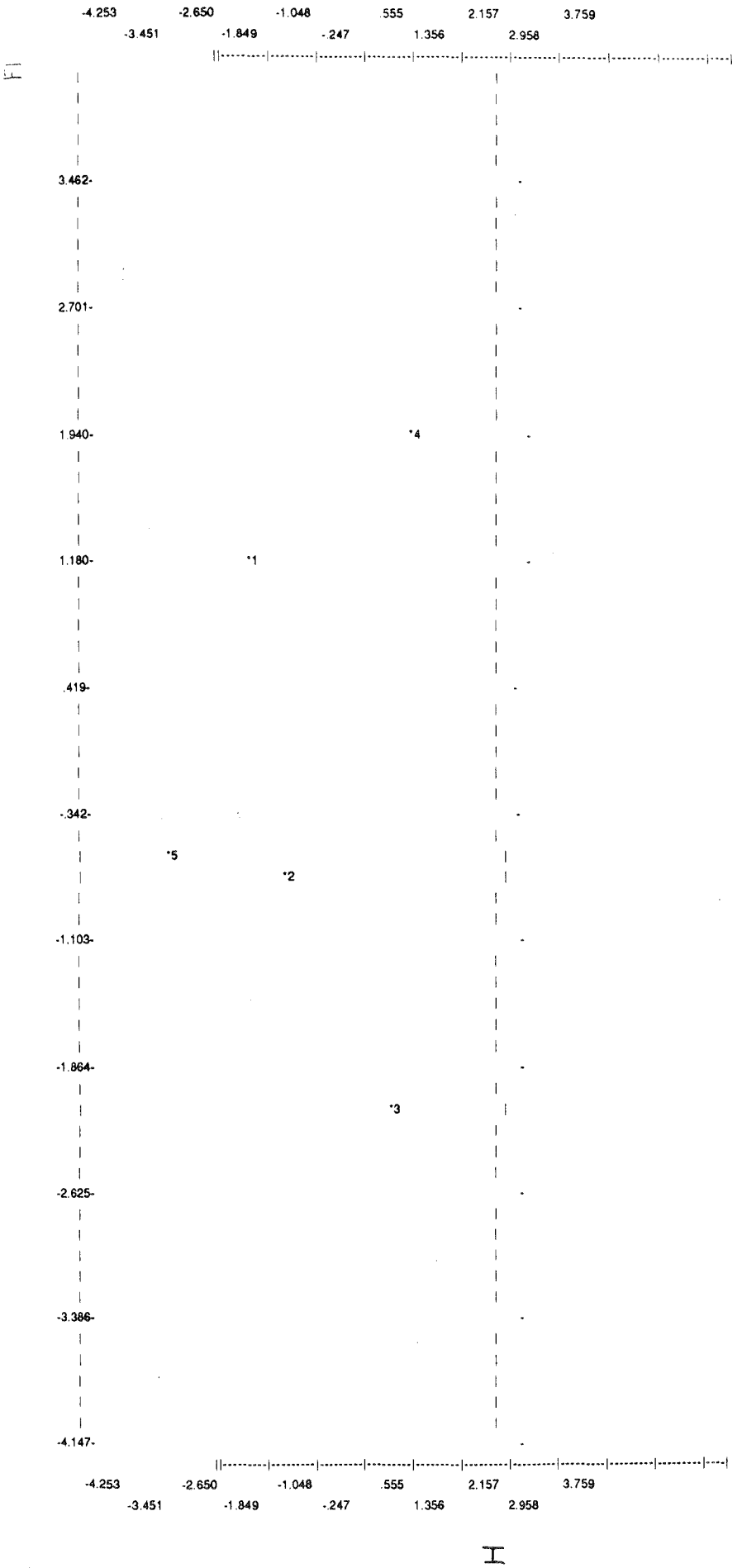
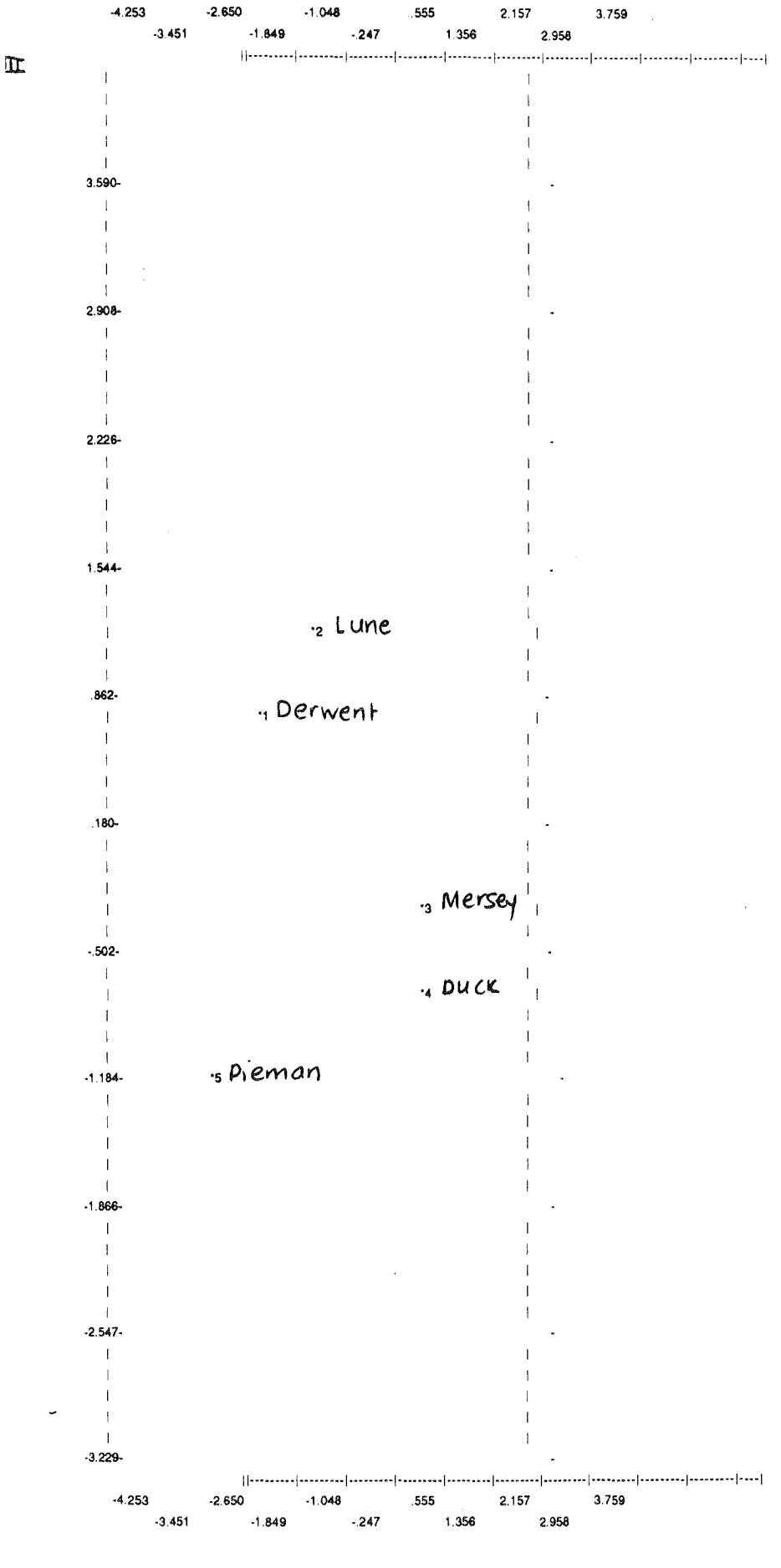


Fig. 3.3 : Group Centroids on two discriminant functions



3.3 Results: *Galaxias maculatus*

3.3.1 Pilot study

Thirty enzymes were surveyed in the pilot study in *G.maculatus*. The following loci were selected for more detailed investigation: *ADA, PEPD, PGD, GPI, GPT* and *PGM*. Gene frequencies at the selected polymorphic loci are shown in Table 3.16. The high levels of polymorphism at locus *ADA* secured its inclusion in the major study. Other loci *PEPD, PGD, PGM, GPI2* and *GPT* were also investigated in order to allow inclusion of as many polymorphic loci as possible to help elucidate population structure of the species. All loci proved easy to score and repeatable results were observed over several electrophoretic runs. The loci were all examined in small samples (N=10 each) of *G. maculatus* collected over a wide geographic range. Due to the small sample size no conclusions could be drawn from the gene frequency data regarding the population structure of the species.

Table 3.16 Gene frequencies (%) of *G. maculatus* in the pilot study

Locus	Allele	Site			
		Gt For	Duck	P	Dwt
ADA	6		5	5	5
	5	5	5	15	10
	4		5	5	
	3	65	65	40	70
	2	25	15	30	10
	1	5	5	5	5
FUM	2	95	95	100	90
	1	5	5		10
GPD	4			5	
	3			5	
	2	95	95	90	95
	1	5	5		5
GPI2	3	5			15
	2	95	95	100	85
	1		5		
GPT	3		5	5	5
	2		15	20	5
	1	100	80	75	90
PEPD	3	10			
	2	90	85	100	95
	1		15		5
PGD	2	95	100	95	100
	1	5	5		
PGM	2	95	100	90	100
	1	5			

3.4 Main Study

Following the pilot study over eight hundred specimens of the species *G. maculatus* were surveyed electrophoretically to determine the spatial stability of allele frequencies at selected gene loci. The samples were collected over a wide geographic range including several states of Australia (Tasmania, Victoria and Western Australia) and representatives of the North and South Islands of New Zealand

3.4.1 Tests for random mating

Allele frequencies have been calculated for each of the polymorphic loci in all samples and are shown in Table 3. 17, together with the corresponding sample size. Low level polymorphism (the frequency of the most common allele ≥ 0.95 in all samples) was observed at loci *GPT* and 6*PGD*. Loci displaying higher polymorphism (the frequency of the most common allele ≤ 0.9 in at least one sample) were *GPI, PGM, ADA* and *PEPD*. None of the loci examined were monomorphic at all sample sites, and no sample populations had identical allele frequencies.

The weighted mean allele frequencies are shown in Table 3.19. In addition a chi-square test for homogeneity of allele frequencies among samples is presented for each allele in Table 3.19. A heterogeneity chi-square value has been calculated for each allele. For example the most heterogeneity across samples was seen at the *ADA* locus. The *ADA* alleles *2* and *3* demonstrated the most significant heterogeneity.

The chi-square tests on the specific alleles proved significant in the alleles listed in Table 3.20 and the the relative contribution of the different samples are tabulated. The results suggest that most of the heterogeneity attributed to the *ADA* alleles *2* and *3* arose in the WA sample. *PGM*4* contributes most to the variance observed at the *PGM* locus and is most of the heterogeneity arose from the Buller River sample from New Zealand. The allele *PEPD*5* present in both New Zealand populations contributes to the heterogeneity observed at this allele across the samples.

A summary of F-statistic values at all loci are listed in Table 3.24. Heterogeneity in the frequency of *ADA* alleles contributed most to the mean F_{ST} value. Heterozygote deficiency designated by (F_{IS}) was also highest at the *ADA* locus (0.171). The total fixation index (F_{IT}) ranged from 0.057 to 0.286 at *GPI2* and *ADA* loci respectively.

Table 3.24 Summary of F-statistics at all loci

Locus	F(IS)	F(IT)	F(ST)
GPI2	0.013	.057	.044
GPT	0.067	.086	.020
6PGD	0.126	.130	.005
PGM	0.059	.093	.036
ADA	0.171	.286	.139
PEPD	0.059	.087	.029
Mean	0.109	.190	.091

Table 3.17 Allele Frequencies for biochemical genetic loci in Galaxias maculatus

Locus	Allele	Site								
		Tas Dwt86	Tas Dwt87	Tas Grt.For	Tas P	Tas Duck	Vic	WA	NZ Ashley	NZ Buller
(N)		100	100	100	95	90	100	48	100	65
Gpi-2										
	1	0	0	0.005	0	0	0	0	0	0
	2	0.050	0.015	0.015	0.021	0.011	0.055	0	0.010	0.008
	3	0.905	0.915	0.945	0.932	0.956	0.890	0.792	0.990	0.969
	4	0.040	0.070	0.035	0.047	0.033	0.055	0.208	0	0.023
	5	0.005	0	0	0	0	0	0	0	0
Gpt										
	1	0.965	1.000	0.990	1.000	1.000	0.990	1.000	1.000	1.000
	2	0.035	0	0.010	0	0	0.050	0	0	0
	3	0	0	0	0	0	0.050	0	0	0
6Pgd										
	1	0.005	0	0	0	0	0	0	0	0
	2	0	0	0.005	0	0	0.005	0	0.005	0.008
	3	0.995	1.000	0.990	1.000	1.000	0.995	1.000	0.995	0.992
	4	0	0	0.005	0	0	0	0	0	0
Pgm										
	1	0	0	0.010	0	0	0	0	0	0
	2	0.020	0.025	0.015	0.032	0.017	0.020	0	0.020	0.023
	3	0.965	0.975	0.965	0.926	0.983	0.980	1.000	0.900	0.830
	4	0.010	0	0.005	0.042	0	0	0	0.070	0.131
	5	0.005	0	0.005	0	0	0	0	0.010	0.008
	6	0	0	0	0	0	0	0	0	0.008
Ada										
	1	0	0.005	0.005	0.026	0.006	0	0.010	0.030	0.015
	2	0.200	0.203	0.150	0.147	0.133	0.250	0.792	0.120	0.123
	3	0.685	0.740	0.740	0.700	0.750	0.638	0.198	0.770	0.746
	4	0.080	0.045	0.090	0.116	0.111	0.112	0	0.065	0.100
	5	0.035	0.005	0.015	0.011	0	0	0	0.015	0.015
Pep-D										
	1	0	0.005	0.005	0.011	0.006	0	0	0	0
	2	0.045	0.015	0.010	0.005	0.039	0.050	0.010	0.005	0.023
	3	0.910	0.965	0.955	0.926	0.928	0.910	0.833	0.890	0.885
	4	0.045	0.015	0.003	0.005	0.028	0.040	0.156	0.030	0.015
	5	0	0	0	0.053	0	0	0	0.075	0.077

KEY:

Dwt86 = Derwent River , TAS 1986
G.F. = Great Forester River, TAS 198
WA = Esperance River, WA 1987
Buller = Buller River, New Zealand 1987
Dwt87 = Derwent River, TAS 1987
Duck = Duck River, TAS 1987
P. = Prosser River, TAS 1987
Ashley = Ashley River, New Zealand 1987
Vic = Barwon River, VIC 1987

Weighted mean allele frequencies and analysis of variation in allele frequencies

Table 3.19: Chi-square analysis of allele frequencies

Locus	Weighted mean	Chi-square	Probability	Heterogeneity Chi-square	df	Probability
GPI2						
N	798		(8df)			
1	0.001	6.98	0.5383			
2	0.023	23.45	0.0028			
3	0.927	50.43	0.0001			
4	0.049	68.85	0.0001			
		6.98	0.5383	106.03	32	0.0001
GPT						
N	798		(8df)			
1	0.993	30.57	0.0002			
2	0.006	33.30	0.0001			
3	0.001	6.98	0.5383	40.28	16	0.0007
6PGD						
N	798		(8df)			
1	0.001	6.98	0.5383			
2	0.003	5.07	0.7504			
3	0.996	5.38	0.7165			
4	0.001	6.98	0.5383	19.03	24	0.7501
PGM						
N	798		(8df)			
1	0.001	13.98	0.0824			

Table 3.19 cont.

2	0.020	3.92	0.8624			
3	0.949	67.44	0.0001			
4	0.026	95.99	0.0001			
5	0.003	7.05	0.5309			
6	0.001	11.28	0.1861	133.05	40	0.0001
ADA						
N	798		(8df)			
1	0.017	25.23	0.0014			
2	0.200	234.52	0.0001			
3	0.695	125.29	0.0001			
4	0.077	20.35	0.0091			
5	0.011	16.84	0.0318	286.16	32	0.0001
PEPD						
N	798		(8df)			
1	0.003	6.71	0.568			
2	0.023	20.95	0.0073			
3	0.917	23.21	0.0031			
4	0.034	52.69	0.0001			
5	0.022	77.07	0.0001	155.32	32	0.0001
Total				739.87	176	0.0001

Table 3.20: Contribution of subpopulations to structuring

Allele		Chi-square	Prob.		Relative contributions to total chi-square							
				Dwt 86	Duck	Grt For	Prosser	WA	Ashley	Buller	VIC	Dwt 87
GPI2	2	23.454	0.0028	0.291	0.046	0.022	0.001	0.094	0.061	0.056	0.407	0.022
GPI2	3	50.430	0.0001	0.029	0.042	0.018	0.001	0.520	0.231	0.067	0.082	0.009
GPI2	4	68.845	0.0001	0.005	0.014	0.012	0.000	0.783	0.149	0.027	0.002	0.028
GPT	1	30.568	0.002	0.755	0.041	0.009	0.043	0.022	0.045	0.030	0.009	0.045
GPT	2	33.301	0.0001	0.796	0.034	0.013	0.036	0.018	0.038	0.025	0.002	0.038
PGM	3	67.436	0.0001	0.016	0.066	0.016	0.029	0.077	0.144	0.549	0.060	0.042
PGM	4	95.593	0.0001	0.022	0.051	0.037	0.019	0.027	0.155	0.577	0.056	0.056
ADA	1	25.228	0.0014	0.137	0.056	0.068	0.040	0.010	0.081	0.001	0.541	0.068
ADA	2	234.517	0.001	0.000	0.021	0.013	0.014	0.897	0.034	0.020	0.000	0.000
ADA	3	125.294	0.0001	0.001	0.021	0.015	0.000	0.892	0.043	0.013	0.000	0.015
ADA	4	20.355	0.0091	0.001	0.142	0.022	0.194	0.395	0.021	0.046	0.035	0.143
ADA	5	16.845	0.0318	0.596	0.122	0.014	0.001	0.065	0.014	0.011	0.133	0.042
PEPD	2	20.947	0.0073	0.201	0.094	0.073	0.129	0.033	0.139	0.000	0.303	0.028
PEPD	3	23.207	0.0031	0.006	0.011	0.162	0.009	0.384	0.085	0.079	0.006	0.259
PEPD	4	52.694	0.0001	0.013	0.005	0.002	0.092	0.812	0.002	0.027	0.003	0.043
PEPD	5	77.067	0.0001	0.058	0.052	0.058	0.108	0.028	0.341	0.238	0.058	0.058

A list of the number of individuals observed at each genotypic class have been generated. The expected number of individuals by the Hardy-Weinberg rule have been calculated. The total chi-square accumulated over all genotypes together with the degrees of freedom and its significance was analysed. The results of this analysis have not been included as the chi-square values are greatly exaggerated due to the small size of some genotypic classes and this procedure is also suspect when the expected numbers in classes are less than one. The BIOSYS-1 program performs a correction for small sample size employed in chi-square analyses. The results of the chi-square test with pooling are given in Table 3.18. Observed and expected numbers of the following three data classes are presented: homozygotes for the most common allele, common/rare heterozygotes and rare homozygotes + other heterozygotes.

As an example the Derwent/86 sample at locus *GPI2* reveals an excess of rare homozygote and other (not common) heterozygote genotypes (observed 3 expected 0.859) and corresponding deficiency in heterozygotes (observed 13 expected 17.28). When the data from the 3 classes is tested for significance using exact probabilities the test reveals a significant deveiation from Hardy-Weinberg equilibrium. In contrast, the Duck River sample at locus *GPI2* reveals significant deviation from Hardy-Weinberg equilibrium even after pooling (P=0.026) but not once the classes are tested using exact probabilities (P=0.150).

The Prosser and Ashley Rivers displays a deficiency in heterozygote classes at the *PEPD* locus and excess rare homozygote genotypes. The same situation is seen in the Victorian samle at the *ADA* locus.

The following genotypic class frequencies were tested for conformance to those excepted under Hardy-Weinberg equilibrium eg. 1: Homozygotes for the most common allele 2: Common/rare heterozygotes and 3: Rare homozygotes and other heterozygotes. The chi-squared goodness-of-fit was performed and the level of significance (P value) determined. Rare genotypes contributed excessively to the X^2 values observed. This is due to the their very small sample size. However, Richardson *et al* (1986) have emphasised that it is essential that the expected frequencies in each class should not be too small. They suggest that the rule of thumb to use is that fewer than 20% of cells should have an expected frequency of less than 5, and no cell should have an expected frequency of less than 1. As an example the Derwent/86 sample at locus *GPI2* reveals an excess of rare homozygotes and other (not common) heterozygote genotypes (observed = 3 and expected = 0.895). A similar situation was observed in the Duck population at the same locus. In the later a single observed individual has had an excessive contribution to the X^2 value together with an expected frequency in that genotypic class of 0.156. This is clearly biologically meaningless. In order to reduce the bias of very small sample sizes the data was then subjected to further analysis using Fisher's Exact Probability Test.

The limitations of statistical tests when an allele is so rare (or sample sizes so small) that only a few copies are expected in each sample, then the sampling distributions given by the null hypothesis (identical allele frequencies in all subpopulations sampled) for the X^2 values do

upon the chi-squared analysis. Richardson *et al* (1986) suggest that the chi-squared test has severe limitations. It is a rather weak test of the null hypothesis as the divergence from the predicted results expected under even quite strong inbreeding are still quite small (i.e. the probability of a type 2 error is large). Secondly, there are, commonly one or more cells with small sample numbers. For this test to be effective very large sample sizes are needed. The sample size can be increased if the results for different sample sets could be combined. Unfortunately combining sample sets will cause a Wahlund Effect if the underlying allele frequencies of the sections of the population sampled are different, though it does not matter if the sample set allele frequencies (i.e. the estimates of the population allele frequencies) are different. The consequence may be to confound the effect to be measured with the effects caused by the measuring technique. Alternatively the chi-squared statistics could be combined. Unfortunately this test does not discriminate between too many and too few heterozygotes and combining the measures does not give a biologically meaningful measure of divergence from equilibrium.

Table 3.18 Chi-square test with pooling for deviation from Hardy-Weinberg equilibrium

Population: DWT86 (G1)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	84	81.859			
	Common/rare heterozygotes	13	17.281			
	Rare homozygotes and other heterozygotes	3	0.859	6.450	1	.011
PGM	Homozygotes for most common allele	93	93.106			
	Common/rare heterozygotes	7	6.789			
	Rare homozygotes and other heterozygotes	0	0.106	0.112	1	.738

ADA	Homozygotes for most common allele	46	46.814			
	Common/rare heterozygotes	45	43.372			
	Rare homozygotes and other heterozygotes	9	9.814	0.143	1	.706
PEPD	Homozygotes for most common allele	82	82.769			
	Common/rare heterozygotes	18	16.462			
	Rare homozygotes and other heterozygotes	0	.769	0.920	1	.338

Significance test using exact probabilities

****

Population: DWT86

Locus	R1	R2	R3	P
GPI2	84	13	3	.039
GPT	93	7	0	1.000
6PGD	99	1	0	1.000
PGM	93	7	0	1.000
ADA	46	45	9	.818
PEPD	82	18	0	1.000

Chi-square test with pooling

****

Population: DUCK

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	83	82.156			
	Common/rare heterozygotes	6	7.687			
	Rare homozygotes and other heterozygotes	1	.156	4.928	1	.026
ADA	Homozygotes for most common allele	49	50.531			

	Common/rare heterozygotes	37	33.939			
	Rare homozygotes and other heterozygotes	4	5.531	.746	1	.388
PEPD	Homozygotes for most common allele	77	77.436			
	Common/rare heterozygotes	13	12.128			
	Rare homozygotes and other heterozygotes	0	.436	.501	1	.479

Significance test using exact probabilities

****

Population: DUCK

Locus	R1	R2	R3	P
GPI2	83	6	1	.150
PGM	87	3	0	1.000
ADA	49	37	4	.572
PEPD	77	13	0	1.000

Chi-square test with pooling

****

Population: GRTFOR

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	90	89.276			
	Common/rare heterozygotes	9	10.447			
	Rare homozygotes and other heterozygotes	1	.276	2.101	1	.147
6PGD	Homozygotes for most common allele	98	97.015			
	Common/rare heterozygotes	1	2.970			
	Rare homozygotes and other heterozygotes	1	.015	65.665	1	.000
PGM	Homozygotes for					

	most common allele	94	93.106			
	Common/rare heterozygotes	5	6.789			
	Rare homozygotes and other heterozygotes	1	.106	8.062	1	.005
ADA	Homozygotes for most common allele	57	54.663			
	Common/rare heterozygotes	34	38.673			
	Rare homozygotes and other heterozygotes	9	6.663	1.484	1	.223
PEPD	Homozygotes for most common allele	93	91.181			
	Common/rare heterozygotes	5	8.638			
	Rare homozygotes and other heterozygotes	2	.181	19.861	1	.000

Significance test using exact probabilities

****

Population: GRTFOR

Locus	R1	R2	R3	P
GPI2	90	9	1	.252
GPT	98	2	0	1.000
6PGD	98	1	1	.015
PGM	94	5	1	.103
ADA	57	34	9	.296
PEPD	93	5	2	.009

Chi-square test with pooling

****

Population: PROSSER

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	82	82.413			

	Common/rare heterozygotes	13	12.175			
	Rare homozygotes and other heterozygotes	0	.413	.471	1	.493
PGM	Homozygotes for most common allele	83	81.481			
	Common/rare heterozygotes	10	13.037			
	Rare homozygotes and other heterozygotes	2	.481	5.525	1	.019
ADA	Homozygotes for most common allele	48	46.444			
	Common/rare heterozygotes	37	40.111			
	Rare homozygotes and other heterozygotes	10	8.444	0.580	1	.446
PEPD	Homozygotes for most common allele	84	81.481			
	Common/rare heterozygotes	8	13.037			
	Rare homozygotes and other heterozygotes	3	.481	15.198	1	.000

Significance test using exact probabilities

****

Population: PROSSER

Locus	R1	R2	R3	P
GPI2	82	13	0	1.000
PGM	83	10	2	.072
ADA	48	37	10	.469
PEPD	84	8	3	.006

Population: WA

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PGM	Homozygotes for most common allele	81	80.955			
	Common/rare heterozygotes	18	18.090			
	Rare homozygotes and other heterozygotes	1	.955	.003	1	.959
ADA	Homozygotes for most common allele	31	30.000			
	Common/rare heterozygotes	14	16.000			
	Rare homozygotes and other heterozygotes	3	2.000	.783	1	.376
PEPD	Homozygotes for most common allele	32	33.263			
	Common/rare heterozygotes	16	13.474			
	Rare homozygotes and other heterozygotes	0	1.263	1.785	1	.182

Significance test using exact probabilities

****

Population: WA

Locus	R1	R2	R3	P
GPI2	29	18	1	.661
6PGD	99	1	0	1.000
PGM	81	18	1	1.000
ADA	31	14	3	.395
PEPD	32	16	0	.323

Chi-square test with pooling

****

Population: ASHLEY

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PGM	Homozygotes for most common allele	45	45.628			
	Common/rare heterozygotes	19	17.744			
	Rare homozygotes and other heterozygotes	1	1.628	.340	1	.560
ADA	Homozygotes for most common allele	59	59.201			
	Common/rare heterozygotes	36	35.598			
	Rare homozygotes and other heterozygotes	5	5.201	.013	1	.909
PEPD	Homozygotes for most common allele	81	79.161			
	Common/rare heterozygotes	16	19.678			
	Rare homozygotes and other heterozygotes	3	1.161	3.644	1	.056

Significance test using exact probabilities

****

Population: ASHLEY

Locus	R1	R2	R3	P
GPI2	98	2	0	1.000
6PGD	64	1	0	1.000
PGM	45	19	1	1.000
ADA	59	36	5	1.000
PEPD	81	16	3	.090

Chi-square test with pooling

****

Population: BULLER

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	61	61.047			
	Common/rare heterozygotes	4	3.907			
	Rare homozygotes and other heterozygotes	0	.047	.049	1	.825
GPT	Homozygotes for most common allele	99	98.005			
	Common/rare heterozygotes	0	1.990			
	Rare homozygotes and other heterozygotes	1	.005	199.00	1	.000
ADA	Homozygotes for most common allele	38	36.093			
	Common/rare heterozygotes	21	24.814			
	Rare homozygotes and other heterozygotes	6	4.093	1.575	1	.209
PEPD	Homozygotes for most common allele	53	51.248			
	Common/rare heterozygotes	13	16.504			
	Rare homozygotes and other heterozygotes	3	1.248	3.262	1	.071

Significance test using exact probabilities

****

Population: BULLER

Locus	R1	R2	R3	P
GPI2	61	4	0	1.000
GPT	99	0	1	.005
6PGD	99	1	0	1.000
PGM	97	2	1	.030
ADA	38	21	6	.321
PEPD	53	13	3	.103

Chi-square test with pooling

****

Population: VIC

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	80	79.161			
	Common/rare heterozygotes	18	19.678			
	Rare homozygotes and other heterozygotes	2	1.161	.759	1	.384
ADA	Homozygotes for most common allele	47	39.744			
	Common/rare heterozygotes	31	45.513			
	Rare homozygotes and other heterozygotes	20	12.744	10.085	1	.001
PEPD	Homozygotes for most common allele	82	82.769			
	Common/rare heterozygotes	18	16.462			
	Rare homozygotes and other heterozygotes	0	.769	.920	1	.338

Significance test using exact probabilities

****

Population: VIC

Locus	R1	R2	R3	P
GPI2	80	18	2	.327
PGM	96	3	1	.049
ADA	47	31	20	.002
PEPD	82	18	0	1.000

Chi-square test with pooling
Population: DWT87

Locus	Class	Observed Frequency	Expected frequency	Chi-square	DF	P
GPI2	Homozygotes for most common allele	83	83.683			
	Common/rare heterozygotes	17	15.633			
	Rare homozygotes and other heterozygotes	0	.683	.809	1	.369
PEPD	Homozygotes for most common allele	93	92.141			
	Common/rare heterozygotes	6	7.719			
	Rare homozygotes and other heterozygotes	1	.141	5.639	1	.018

Significance test using exact probabilities
****
Population: DWT87

Locus	R1	R2	R3	P
GPI2	83	17	0	1.000
PGM	96	3	1	.049
PEPD	93	6	1	.135

3.4.2 G-tests

3.4.2.1 Comparisons between individual samples

G-tests were performed on all possible pairs of populations. The detailed results are found in Table 3.22. Of the 36 comparisons between populations, 24 were found to be statistically significant.

No temporal heterogeneity was observed in samples collected from the Derwent River in successive years (1986 and 1987) on the basis of comparisons of allele frequencies when analysed using the G-test.

Table 3.22 G-tests between all *G. maculatus* populations

OTU 1	OTU 2	Gstat	D of F	Prob.
Ashley	Buller	15.16	19	0.71
	WA	222.93	18	*
	Duck	66.57	20	*
	Dwt/86	82.88	21	*
	Prosser	29.48	19	0.05
	Grt For	60.04	23	*
	VIC	78.97	18	*
	Dwt/87	79.23	19	*
Buller	WA	195.78	19	*
	Duck	61.33	21	*
	Dwt/86	71.65	22	*
	Prosser	23.61	20	0.25
	Grt For	58.71	24	*
	VIC	77.06	19	*
	Dwt/87	72.38	20	*
WA	Duck	174.88	16	*
	Dwt/86	162.45	20	*
	Prosser	190.23	17	*
	Grt For	175.42	23	*
	VIC	154.53	15	*
	Dwt/87	141.84	16	*
Duck	Dwt/86	37.16	20	0.11
	Prosser	41.08	18	.001*
	Grt For	19.85	23	0.65
	VIC	22.63	16	0.12
	Dwt/87	15.83	16	0.46
Dwt/86	Prosser	63.98	21	*
	Grt For	28.81	24	0.22
	VIC	40.74	20	.004*
	Dwt/87	36.93	20	0.011
Prosser	Grt For	42.19	23	.008*
	VIC	56.56	18	*
	Dwt/87	41.37	17	.008*
Grt For	VIC	39.14	22	0.013
	Dwt/87	22.53	22	0.428
VIC	Dwt/87	27.05	16	0.04

The G-statistic data suggest three major genetic pools of *G. maculatus*. The first includes the Prosser River from the east coast of Tasmania together with the Ashley and Buller Rivers of New Zealand's south island. The second grouping consists of northern Tasmanian rivers (Duck and Great Forester) together with Victoria. This group is also linked to the Derwent River samples collected in the South-east of Tasmania.

Data from the allele freqencies from the *ADA*, *GPI* and *PGM* loci together with G-statistic results all indicate that *G. maculatus* from the Esperance River in Western Australia represent a third and unique genetic pool. It is interesting to note that the populations represented in by the first genetic pool were significantly different to both Derwent River samples. The geographic proximity of the Prosser and Derwent rivers may have predicted a common genetic pool. On the basis of G-statistic data the Derwent River fish appear to maintain genetic links with the northern Tasmanian populations of the Duck and Great Forester rivers. Northern Tasmanian fish captured in 1986 were not significantly different to the 1986 or 1987 Derwent specimens, suggesting a degree of genetic stability: Victorian fish although significantly different to the 1986 Derwent sample were not significantly different to fish caught in 1987. At present the results do not lead to any clear conclusions about the relationship between Derwent River fish and their northern neighbours. These fluctuations in genetic relatedness suggest that it would be useful to both increase the sample size and extend the study over a longer period of time.

The locus which contributes most to the differentiation of the West Australian genetic pool is *ADA*. There is a marked discontinuity at this locus between Western Australian and all other populations.

3.4.2.2 G_{ST} analysis of pooled data

Nei's gene diversity statistic (G_{ST}) listed in Table 3.25 illustrates the extent of the genetic differentiation among samples. The $P<0.05$ values indicate significant differentiation between samples that cannot be explained by sampling error alone. With the exception of loci *GPT* and *6PGD* all other loci contribute to the genetic differentiation observed in *G. maculatus*. The G_{ST} values range from 0.0045 at the *6PGD* locus where differentiation may be attributed to sampling error ($P=0.465$) to 0.1394 ($P<0.001$) at the *ADA* locus.

Table 3.25 G_{ST} analysis of *G. maculatus* samples

Locus	G_{ST}	G_{STnull}	SD of G_{STnull}	*P* value
GPI2	0.0444	0.0054	0.0021	< 0.001
GPT	0.0200	0.0049	0.0024	0.001
6PGD	0.0045	0.0047	0.0016	0.465
PGM	0.0365	0.0048	0.0019	< 0.001
ADA	0.1394	0.0054	0.0021	< 0.001
PEPD	0.0293	0.0053	0.0019	< 0.001

3.4.3 Nei's genetic distance (D) and identity (I)

Nei's genetic distance (D*) and genetic identity (I) are shown in Table 3.23 . The D* values range from 0.0005 between successive samples from the Derwent River to 0.1165 between Western Australia and the Buller River, New Zealand. The results clearly highlight the genetic difference of the Western Australian population from all others studied. It also illustrates the close relationship of all other samples appear to be on the basis of genetic identity and distance. For example, the Derwent River populations do not appear genetically isolated on the basis of their genetic distance or identity from any other population with the exception of Western Australia.

An unrooted matrix of association was produced using Nei's genetic distance data. The dendrogram clearly illustrates that the difference between the West Australian population is greater than the differences between any of the other populations. The dendrogram illustrates the close genetic relationship of the rivers of New Zealand's east and west coast. The Derwent and Victorian samples have been grouped together. Northern Tasmanian rivers and the Prosser also form a group based on the Nei's distance data. On the basis of the G-statistic tests it may have been expected that the Prosser River would have appeared to be more closely related to the New Zealand samples than shown in Fig 3.5. The Nei genetic distances are very small between all samples with the exception of Western Australia. It would be imprudent to over split the samples and the data clearly shows that with the exception of the WA outgroup the other geographic regions appear to share a common genetic pool.

Table 3.23: Nei Genetic Identity (I) and Distance (D)

D→ above diagonal

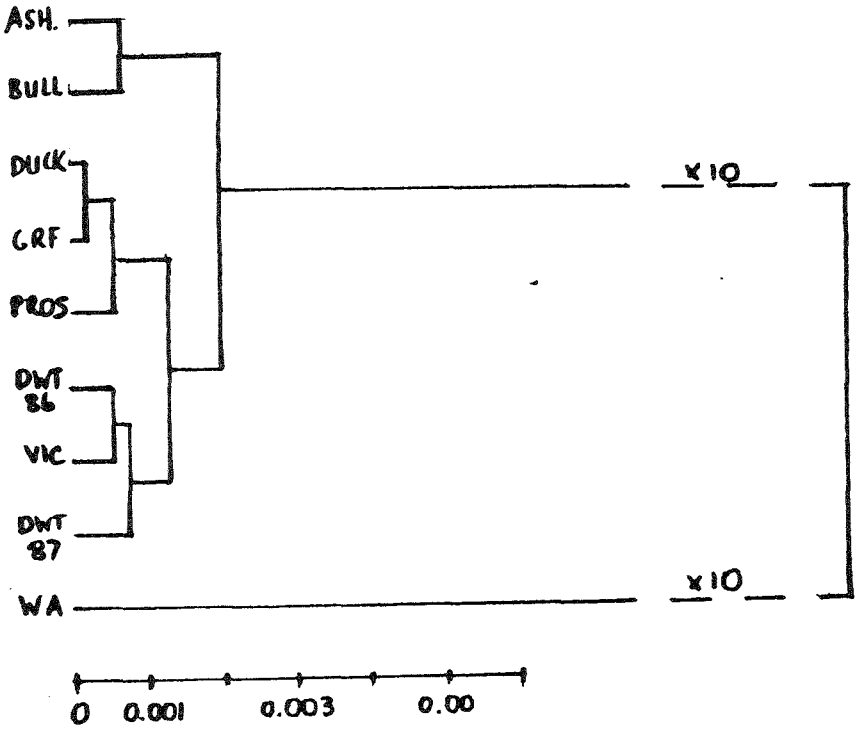
I ↓ below diagonal

		1	2	3	4	5	6	7	8	9
1	Ashley		0.0007	0.1122	0.0009	0.0019	0.0008	0.0007	0.0018	0.0020
2	Buller	0.9993		0.1165	0.0021	0.0034	0.0015	0.0021	0.0037	0.0037
3	WA	0.8938	0.8900		0.1115	0.0935	0.1053	0.1070	0.0963	0.0941
4	Duck	0.9991	0.9979	0.8945		0.0013	0.0006	0.0002	0.0015	0.0016
5	Derwent/1	0.9981	0.9966	0.9107	0.9987		0.0013	0.0009	0.0005	0.0006
6	Prosser	0.9992	0.9985	0.9001	0.9994	0.9987		0.0005	0.0014	0.0014
7	GrtFor	0.9993	0.9979	0.8986	0.9998	0.9991	0.9995		0.0011	0.0009
8	Vic	0.9982	0.9964	0.9082	0.9985	0.9995	0.9986	0.9989		0.0008
9	Derwent/2	0.9980	0.9963	0.9102	0.9984	0.9994	0.9986	0.9991	0.9992	0.0000

FIG 3.5 Unrooted tree for G. maculatus derived from Nei's genetic distance data

Key

ASH	Ashely River, NZ
BULL	Buller River, NZ
DK	Duck River, Tasmania
GRF	Great Forester River, Tasmania
PROS	Prosser River, Tasmania
86DWT	Derwent River, Tasmania (1986)
VIC	Barwon River, Victoria
87DWT	Derwent River, Tasmania (1987)
WA	Esperonce River, Western Australia



Chapter 4

4.0 Discussion

4.1 Genetic differentiation among populations

The electrophoretic survey of selected Tasmanian "whitebait" species *Lovettia sealii* and *Galaxias maculatus* revealed genetic heterogeneity within both species. *L. sealii* is a species endemic to the small geographic area of Tasmania. Prior to this study *L. sealii* stocks were considered to be derived from two genetic pools: one in the north of Tasmania and the other in the south (Blackburn, 1950). The present survey revealed that the situation is far more complex than a simple north - south divide, with individual rivers capable of supporting unique stocks. A similar degree of genetic heterogeneity was observed in the globally dispersed species *G. maculatus*. As both species are the major components of potentially commercial "whitebait" runs, an understanding of the population structure of both species will assist in the prudent management of this resource. At present, this resource is managed as a single unit but with very restricted public access and no commercial exploitation. The pattern of genetic variation reflects an underlying stock structure that will need to be considered in the management of this species (Parkinson, 1984). The present study was part of a larger 3-year project examining the potential viability of a whitebait fishery in Tasmania but, primarily aimed to collect as much data as possible of the biology of *L. sealii* and associated species which are collectively known as whitebait. The interpretation of the genetic data has been made in the context of the known and observed biology of the species.

The null hypothesis for this study was that all samples of a species were representatives of a single genetic stock. A significant difference would indicate that samples were from different genetic pools.

Life-history, rates of genetic drift, migration and prevailing environmental conditions will all contribute to the genetic structure of fish populations. Unlike electrophoretic surveys of many other fish species, especially the Salmonids (Perkins *et al*, 1993), the genetic variability observed in this study can be attributed to naturally occurring populations. No populations of either species have been subject to stocking from hatcheries or resettlement from other areas. Slatkin (1987) suggested that the overall geographic range of a species is determined largely by a series of historical events. A species will extend its range until it is stopped by barriers to dispersal. On a smaller scale, where a species occurs is determined primarily by ecological factors, including climate, predators, competitors and usable resources. The distribution of the "whitebait" species surveyed is disparate and their origins, in particular that of *G. maculatus,* have previously been the subject of much debate (McDowall, 1978; Croizat, 1974; Rosen, 1974). The electrophoretic survey revealed the presence of genetic heterogeneity in both species but the influence of the above factors on population structure is possibly different.

4.2 *Galaxias maculatus*

Populations of *G. maculatus* collected in several states of Australia consitute at least four genetically divergent groups: a Western Australian group, a group representing the New

Zealand samples. A third group contains populations from the Duck, Prosser and Great Forester Rivers. The fourth grouping with representatives from the Derwent River together with Victoria.

The adenosine transaminase (*ADA*) locus provided the most useful data for the determination of population structure in *G. maculatus*. However, the population sub-structuring does not rely soley upon information obtained at a single locus as the locus *6PGD* was also significantly different between sites.

In this study *G. maculatus* was sampled as juveniles returning to freshwater. In contrast, *L. sealii* was captured as mature adults. Allendorf and Phelps (1981) suggest that it is dangerous to draw conclusions about reproductive isolation between adults by estimating allelic frequencies in their progeny. Differences caused by a small number of reproducing adults without any reproductive isolation can become highly statistically significant when a large number of progeny are sampled. There are, however, a number of precedents for the use of larval samples in establishing the genetic structure of a population; these include Johnston *et al.* (1986) and Johnston *et al.* (1987). *G. maculatus* produce large numbers of eggs, up to 13,500 have been observed in larger females (McDowall, 1968). The small size of the eggs is generally related to high mortality. It is therefore assumed that the contribution of individual fish to the next generation will be low and the samples collected in the study will reflect the genetic structure of the species as a whole.

A comparison of allele frequencies at the *ADA* locus was clearly the most striking result of this study. Sharp clines especially where more than one locus is involved, are often considered to be of major taxanomic significance (Parkinson, 1984). This suggests that that the heterogeneity observed at the other loci is not due to genetic drift following inbreeding. Such a process would affect all loci in a similar manner unless counter-acted by a strong locus-specific stabilising force (Richardson *et al.*, 1987). Significant differences were seen at five of the six polymorphic loci investigated. However, differences even major differences, at a small number of loci should not be taken to represent a fundamental subdivision of a species without corroborating evidence from a wider range of characteristics (Parkinson, 1984). The physical habitat of the localities sampled did not appear to differ sufficiently to generate the genetic differences.

The clearest genetic divergence was observed in *G. maculatus* from Western Australia. On the basis of allele frequency data, Western Australian fish clearly represent a gene pool isolated from Tasmanian, Victorian and New Zealand members of this species. Isolation by distance may occur between populations if gene flow is proportional to geographic distance. The isolation-by-distance model proposed by Wright (1969) assumes that random movements of groups of a species occur and that the only factor limiting the movement between habitable areas is the geographical distance between them. This mechanism could have been the first step in the divergence of the Western Australian population from those of south-eastern Australia and New Zealand. It would be informative to sample areas between the geographic extremes (for example in South Australia) to determine if there is evidence of genetic exchange between Western Australia and South Australia and also the latter with Victoria. The marked allelic discontinuity observed in this study may be due to the sampling of representatives at the extremes of a clinal range. However, it is more likely that the Western Australian population is now a self-sustaining genetic isolate.

The Leeuwin Current off the coast of Western Australia would promote the movement of larvae eastward. Simpson (1991) proposed that the current provides a mechanism for unidirectional gene flow. The Leeuwin Current, originating as inflow from the Western Pacific to the Indian Ocean through the Indonesian Archipelago (Godfrey and Ridge, 1985), flows strongly southward along the coastline during the autumn and winter. Weaker flows and periodic reversals occur during late spring and summer when the south-westerly winds prevail (Simpson, 1991). The Leeuwin Current roughly parallels the East Australian Current which brings warm waters southward to about 35°S before diverting as eddies into the Tasman Sea (Morgan and Wells, 1991). The genetic isolation of the Western Australian *G. maculatus* would be assisted by the Leeuwin as larvae from eastern Australia are unlikely to move across Bass Strait counter to prevailing ocean currents. However, Rochford (1986) suggested that this current could carry stocks of young fish (e.g. Australian salmon) from west Australian to east Australian waters. It is possible that the westward moving Flinders Current off South Australia may reduce the potential of Western Australian *G. maculatus* to access south-eastern Australia. Rising sea levels and increasing temperatures since the last glaciation may also have served to isolate the West Australian population and led to its differentiation from east Australian populations.

On the basis of G-test comparisons and Nei's genetic distance data a second genetic "stock" incorporates Victorian, Tasmanian and New Zealand rivers. In particular, the low level of genetic diversity between Victoria and Tasmania populations may be due to the larval dispersal across Bass Strait. The transfer of several adults each generation should be enough to maintain similarity between distant populations (Hartl, 1980).

During the last glaciation, 10,000-20,000 years ago, a land bridge connected Tasmania to southeast mainland Australia (Davies, 1974). At this time southern Victorian coastal drainages merged with northern Tasmanian drainages before flowing westward into the sea, allowing reproductive contact between Victorian and Tasmanian galaxiids (Ovenden and White, 1990). A survey of mitochondrial DNA diversity in *Galaxias truttaceaus* detected some low-level divergence at the level of mtDNA between Victoria and Tasmania fish. Ovenden and White (1990) postulated that this was a reflection of reduced gene flow due to geographic distance. Divergence may have occurred as a result of the rising sea levels which severed the connection between the coastal drainages. This species of galaxiid exhibits a life-history strategy similar to *G. maculatus*. The larval stage of riverine populations spends 3 months at sea before returning to fresh water. Ovenden and White (1990) proposed that there was an opportunity for genetic exchange between streams during this marine phase. In larval *Mordacia mordax* the very low Nei distance (0.0001) between samples captured in southern Tasmania and Victoria is also thought to imply intermixing of adults between the states (Johnston, 1987). In the present study, a mean genetic distance of 0.0013 was observed between Victorian and Northern Tasmanian *G. maculatus*. The prevailing ocean currents off the northern Tasmanian coast could readily sweep larval fish into Bass Strait and allow for genetic mixing. Slatkin (1987) noted that planktonic larvae of many marine species can survive for months in the ocean and disperse passively with currents. Although tracking an individual larva is not possible, the capacity for long-distance dispersal and the wide geographic range of many marine species suggests that gene flow over long distances is common. This situation could be readily applied to the larval phase of *G. maculatus*. Slatkin (1987) cautioned that the capacity for dispersal does not always predict how much gene flow actually occurs. Passive dispersal of larvae may find the species in an unfavourable environment. McDowall's (1975) observation of larval *G. maculatus* over 700 km from land

illustrates the potential capacity of the larvae for movement between Tasmania and Victoria or Tasmania and New Zealand.

Galaxias maculatus of the Derwent River share genetic ties with Victorian and other Tasmanian rivers except the east coast Prosser River and are distinct from New Zealand populations. This inconsistent pattern may be the result of northern fish moving into southern waters and mixing with Derwent inhabitants. After hatching larval Derwent *G. maculatus* are likely to be carried by the prevailing currents into the lower river reaches and into Storm Bay. High salinity oceanic currents within the bay move in a clock-wise fashion (Cooper *et al.*, 1982). The majority of drifting larvae would be carried to the western coast of the Tasman Peninsula and the circulation pattern in this area would tend to maintain the larvae within the bay. It is possible that as the larvae mature they are attracted to the freshwater discharge from the Derwent River. This would stimulate their migration upstream and hence maintain an isolated genetic unit. However, other larvae may enter the D'Entrecasteaux Channel and hence the lower reaches of southern rivers such as the Huon or Lune. During a rising tide the movement of water from the mouth of the Derwent River into Fredrick Henry Bay via Outer North Head was established by the Electrolytic Zinc Company (1983) in a survey of the hydrology of the Derwent Estuary. The complex hydrology of the area may promote the isolation of larvae and lead to the formation of small genetic isolates as observed in the *L. sealii* of Allen's Creek on the Tasman Peninsula. The west to east movement of brackish waters was confirmed by a drogue study conducted in 1979-80. This results in water from the Derwent River entering adjacent Storm Bay and into Fredrick Henry and Wedge Bays. The mechanism for these water movements appeared to be the large tidal demand of these bays (Cooper *et al.*, 1972).

The homing instinct of salmonid fishes (reviewed in Hasler and Scholz, 1983; Stabell, 1984) provides a behavioural-genetic mechanism for the evolution of reproductively isolated populations over small geographic areas. Do larval *G. maculatus* return to their natal stream for spawning? Do they possess the characteristic homing instinct which is so highly developed in salmonid fishes?

Despite this homing behaviour, most population genetic studies of anadromous salmonid fishes have sampled populations over relatively large geographic areas in an attempt to detect genetically differentiated stocks of fish that can subsequently be identified in mixed-population fisheries (Utter *et al,* 1980; Beacham *et al*, 1985). Fewer studies have intensively sampled populations within relatively small geographic areas. Such intensive surveys can potentially yield information regarding the extent of population structuring on a microgeographic scale (Parkinson, 1984). Such information can further provide insights regarding the evolutionary significance of the homing instinct of these fishes. Tagging studies to determine the preciseness of the homing instinct have never been performed or reported in the published literature. However, tagging studies with salmonid fishes have generally demonstrated homing rates between 90 and 100 % (Stabell, 1984).

Barker and Lambert (1988) investigated the implications of natal river return by *G. maculatus* on the genetic struture of populations. Significant differences were reported for two loci in four diadromous populations of *G. maculatus* from rivers within the Bay of Plenty, New Zealand. However, they concluded that although the degree of differentiation recorded did not support complete natal river return, the result may reflect natural fluctuations due to high larval mortality. Any homing response of *G. maculatus* larvae must take into account that the eggs hatch and are taken out to sea within a few hours of hatching, so that any idea that a

firm attachment with the spawning area could have been formed should be regarded sceptically. (Benzie, 1968). McDowall (1968) confirmed the rapid movement of hatching larvae being washed out to sea with the ebbing tide. In comparison to larger salmonid species the galaxiid juvenilles are small and weak swimmers which would make homing more difficult.

The Fst values determined across all loci in G. maculatus in the present study and that of Barker and Lambert (1988)are very similar. In the present study we observed Fst values to 0.093 at the ADA locus with a mean of 0.062, Barker and Lambert (1988) reported 0.05. Wright (1978) suggests that this represents a moderate degree of interpopulational differentiation. In contrast, Avise and Felley (1979) found the bluegill (*Lepomis machrochirus*) revealed lower level differentiation (Fst = 0.029) within drainages but moderate levels among reservoirs (Fst = 0.392). Brook trout surveyed across a number states in the northeastern United States revealed Fst values of 0.375 (Perkins *et al.*, 1993) which the authors considered that if the survey was extended throughout the entire native range of the species than the level of differentiation would be comparable to that observed by Kornfield *et al.* (1981) in Artic char *(Salvelinus alpinus)* (Fst = 0.533). Very low Fst values (0.072) have been reported in marine species of starfish (*Acanthaster planci*) which span the Pacific Ocean (Nash *et al.*, 1988)

Phelps (1981) and Altukhov *et al.*(1984) have shown that genetic drift among subpopulations can yield significant differences in allele frequencies despite the presence of gene flow. Although Fst attains an equilibrium value when gene flow is restricted, allele frequencies within each subpopulation will nevertheless fluctuate randomly over time due to genetic drift. The magnitude of these fluctuations depends inversely upon the effective size (N) of the subpopulations and the amount of gene flow among them. The population structure may thus appear highly dynamic in terms of allele frequencies for the individual subpopulations, but the overall structure is quite stable in terms of Fst, mean gene frequencies, and average heterozygosities. The net effect is that restricted gene flow can maintain the same alleles within most subpopulations, but may not be sufficient to homogenize allele frequencies among subpopulations. Genetic variation is thus preserved in a subdivided population, relative to a single panmictic population of equal size, because no allele goes to fixation in all subpopulations simultaneously.

High levels of genetic similarity between populations often appear to be associated with high levels of mobility, whether in the adult or larval stages. The extent to which the potential for wide dispersal is realized depends on such factors as local hydrographic conditions and larval behaviour (Nash *et al.*, 1988). The fact that *G. maculatus* is found in South America, New Zealand and Australia may be evidence to support its high dispersal capability. In particular the results of the present study which highlight the genetic relatedness of the species in New Zealand and the east coast of Tasmania. As suggested by McDowall (1978), these populations of *G. maculatus* have diverged very little either due to the recency of their arrival, continuing gene flow, or slow phyletic evolution. McDowall (1978, 1980) advocates that the present distribution of the galaxiid family is probably due to southern, transoceanic dispersal. An alternative view proposed by Rosen (1974) considers the distribution of fishes such as *G. maculatus* to be a consequence of the Mesozoic fragmentation of Gondwana. The later would require that there has been virtually no phyletic evolution since the late Mesozoic (McDowall, 1980). The use of land bridges has also been invoked to explain the dispersal of *G. maculatus* across the southern oceans to reach Australia, South America and the Falkland Islands (Campos, 1974). The Western Australian population confirms the potential for genetic

divergence in this species. Although it is possible for populations to be genetically similar because they are subject to similar selection pressures, the ties between Australian and New Zealand are most likely the result of genetic exchange.

Hartl (1981), Allendorf and Phelps (1981) maintain that only a few migrants per generation are sufficient to inhibit differentiation of populations. Electrophoretic analysis of populations of the lamprey *Geotria australis*, sampled from Western Australia, Tasmania and New Zealand suggested that adults may intermix over distances greater than 4000 km (Johnston *et al.*, 1987). In common with *G. maculatus* this species is found in Australia, New Zealand and South America. In contrast to the present study the genetic distances between the populations of *G. australis* from Tasmania, New Zealand and Western Australia were exteremely small. They ranged from 0.003- 0.009 when calculated on the basis of polymorphic loci. This is not dissimilar to the value obtained between Tasmanian and New Zealand *G. maculatus*, however the genetic distance between these populations of *G. maculatus* and Western Australia was found to in the range 0.09-0.11. The lamprey study would appear to confirm oceanic dispersal as a means of species distribution and maintenance of genetic links. However, the Western Australian *G. maculatus* population does not exploit this potential. Natal return of *G. maculatus* together with local current systems may provide mechanisms which isolate these populations. The pattern of allele frequencies reflects not only potential gene flow between populations but is determined by natural selection, genetic drift and mutation (Slatkin, 1987).

McDowall (1964) suggested that the New Zealand Galaxiidae had Australian origins. Dispersal in the south-temperate region is probably west to east. New Zealand lies about 2000km east of southeastern Australia. Oceanographically, New Zealand intersects the flow of a large apparently irregular gyral system in the Tasman Sea (McDowall, 1978). This results in the movement of parcels of warm, subtropical water from Australian coasts to New Zealand. The East Australian Current may split so that part of it wanders eastward in the general direction of New Zealand, following what is called the Tasman Front (Cresswell, 1987). The apparent genetic isolation which exists between the Victorian and New Zealand samples may be explained by the studies of Hamilton (1990) which show that water masses originating from the east coast of Australia near 34°S move in a north-easterly direction and would pass north of New Zealand. Brasher *et al.* (1992) proposed that the current system explained the genetic subdivision observed between Australian and New Zealand populations of the palinurid rock lobster (*Jasus verreauxi*). Only the more southern waters of the Tasman Sea are thought to impact upon New Zealand. This will afford an opportunity for genetic exchange and support the genetic homogeneity observed between *G. maculatus* from the Tasmanian east coast river (Prosser) and the Buller and Ashley rivers of New Zealand. The west-east movement of fishes from Australia to New Zealand is also supported by comparison of esterase frequencies between Pacific skipjack tuna (*Katsuwonus pelamis*) captured in Australia and New Zealand together with the recovery in New Zealand of fish tagged in Australia (Richardson, 1987). The Westland, Southland and D'Urville currents (Heath, 1985; Vincent *et al.*, 1991) could facilitate genetic exchange between the west (Buller) and east (Ashley) coast rivers of New Zealand's South Island and maintain the very close genetic ties observed in this study. Booth *et al.* (1990) conducted comparisons of the morphology, colour, life-history and genetic composition of rock lobsters of the *Jasus* subgroup *"lalandii"* in New Zealand and south-eastern Australia. They found a lack of genetic heterogeneity and suggested that the known current patterns between New Zealand and Australia together with the widespread occurrence of the larvae across the Tasman Sea justification to reunite the Australian and New Zealand populations into a single species.

Although the *G. maculatus* sample from the Prosser River appears as separate group from the New Zealand samples in the unrooted tree diagram, the genetic distance between them was very small (0.0008 and 0.0015 for the Ashley and Buller respectively) and G-test comparisons did not reveal any genetic divergence.

A large proportion of New Zealand's fish fauna has direct and close Australian affinities Moreland (1958) suggested up to 31% of the species. In New Zealand the largest freshwater family is the Galaxiidae, and 13 species (11 endemic) (McDowall, 1980). Larvae and juvenile of *G. maculatus* , the principal component of the New Zealand whitebait catch, occur in the sea, and adults spawn in the river estuaries. McDowall (1975) found that larvae as small as 4.5-7.0 mm have been collected at sea, which suggests that they go to sea within a few hours of hatching. McDowall (1975) caught larval *G. maculatus* on the Bounty Rise between the Antipodes Islands and Bounty Island about 700 km from New Zealand and 600 km from the Chatham Islands were the species are known to occur. Despite this observation, Rosen's (1974) contention that there is no evidence that galaxiid does undertake, or is capable of undertaking major, transoceanic migrations cannot be equivically disputed. McDowall (1972) stated that it is not known whether the juveniles travel great distances, or whether a small part of the population is swept away from the coast by ocean currents. However, McDowall (1975) considered the observations of larvae at sea to indicate that dispersal via transoceanic routes was possible.

In a major review of the biology of New Zealand *G. maculatus* McDowall (1968) concluded that the difference in the size of migratory whitebait suggested that there was not free mixing of stocks in the seas around New Zealand. McDowall (1968) claimed this was illustrated by a comparison of whitebait catches from the east and west coasts of the South Island. East coast whitebait were consistently shorter than those caught on the west coast. However, it is not clear from his discussion whether the term "whitebait" referred to *G. maculatus* or multiple galaxiid species. The author compares differences in New Zealand whitebait stocks to Blackburn's (1950) conclusions regarding *L. sealii* populations in the north and south of Tasmania. The Buller and Ashley Rivers represent the west and east coasts of the South Island respectively. However, on the basis of G-statistic, Nei's Genetic Distance and Identity in the present study it can be concluded that the two populations are part of a single genetic pool. Not only are the representatives of the South Island part of a common genetic pool but it also extends to the Prosser River on the east coast of Tasmania. The fish captured in the Prosser River could not be distinguished from the New Zealand samples on the basis of G-statistic analysis. The apparent exchange of genetic material between Australia and New Zealand would support McDowall's hypothesis (1966) that the galaxiids have been distributed amongst the southern land masses (Australia, New Zealand and South America) by oceanic routes. Key evidence for the exchange of genetic material across the Tasman Sea lies with the shared rare allele *PEP-D*5* observed in the Prosser, Ashley and Buller rivers. Wehrhahn (1987) in a study of coho salmon concluded that populations with the same rare alleles, for one or more loci, could not have any absolute barriers to dispersal and gene flow. Both Fst and rare alleles can act as indicators of the levels of gene flow. Statistically significant patterns of allelic divergence are often present when there is considerable exchange between sub-populations. The finding of significant allelic divergence should not be interpreted to imply that the amount of genetic exchange is low (Allendorf and Phelps, 1981).

4.3 *Lovettia sealii*

Blackburn (1950) undertook the last major study of *L. sealii* which involved the sexing and measurement of over 78,000 specimens. The present study selected a similar sampling strategy for the collection of specimens for electrophoretic analysis but, included a more comprehensive sampling of southern rivers.

The majority of genetic surveys of fish have concentrated on adult representatives (King *et al.*, 1987; Roby *et al.*, 1991; Shaklee and Salini, 1985) as reccomended by Allendorf and Phelps (1981). Most have endeavoured to investigate a single year class, as multiple classes have led to ambiguous results (Johnston *et al.*, 1986; Parkinson, 1984; Richardson and Habib, 1987). In the case of *L. sealii* all specimens, mature spawning adults, had the capacity to pass their genetic information onto the next generation of the species

The only reference in Blackburn's (1950) study to population structure concluded that northern and southern Tasmanian populations can be distinguished on the basis of differences in growth rate and pigmentation. Blackburn (1950) defined the growth rate "as the average size at the same age", which was found to be variable but greater in the northern material. The pigmentation was observed to be slightly more extensive in southern than in northern fish of the same sex and maturity stage. Blackburn (1950) concluded that it was not possible to distinguish populations by measurable morphological characters. Morphometric and meristic characters were also not found to be useful in separating populations in the present study.

The contention that all northern rivers were part of a single stock was explained on the basis that *L. sealii* larvae were carried out to sea where this species prolonged marine phase would provide ample opportunity for mixing of larvae from other rivers. Further evidence of homogeneity in the north of the state was proposed from catch records. The uniform decline in catch across all northern rivers (except the Forester) in 1948 compared to 1946 and 1947 suggested a decline in the entire northern pool. Although not subject to the same fishing pressure, there was no apparent decline in southern whitebait comparable to that experienced in the north. This data would also support separate northern and southern *L. sealii*. The north-south geographic differentiation of *L. sealii* in Tasmania may have resulted from a simple process of isolation by distance where gene exchange occurs preferentially between neighbouring populations.

In common with steelhead (*Salmo gairdneri*) in British Columbia (Parkinson, 1984), *L. sealii* appears to be subdivided into a large number of semi-isolated populations that differ genetically to varying degrees but still maintain basic morphological and biological similarity which warrant their inclusion in a single species.

The stability of allele frequencies over three successive spawning seasons was studied in *L. sealii*. With the exception of the Mersey River temporal stability of allele frequencies allowed the data to be used as characteristic of a locality rather than characteristic of a collection (Salini and Shaklee, 1987).

Electrophoretic data in the present study support a multi-stock model for northern Tasmanian *L. sealii*. The morphometric analysis would support separate stocks in all five rivers that were examined: Mersey, Lune, Derwent, Pieman and Duck. Nei's data separates the species along the following lines: firstly the broad isolation of north from south Tasmania, then smaller sub-units from selected regions of the Tasman Peninsula, Pieman River on the west coast and the Derwent River/Allen's Creek group as a sub-group of southern *L. sealii*.

Within-river variability in the Mersey in 1985 as well as the variation between seasons illustrates the complexity of the population sub-structuring of the species. A clearer difference between northern rivers is seen in 1986 where on the basis of allele frequency data the Duck, Mersey and Leven Rivers all appear to support separate stocks of *L. sealii*. These localised populations may be largely self-sustaining, with consequent incomplete gene flow (Nash *et al.*, 1988). Limited information is available about the hydrology of Tasmania's coastal rivers and the movement of water into Bass Strait. Larvae may enter river estuaries and maintain their genetic integrity by schooling in the outer estuary. There is only anecdotal evidence of schooling "whitebait" observed at sea (Blackburn, 1950).

In parallel to the east-west geographic differentiation of capelin, *Mallotus villosus* in the Gulf of St Lawrence (Roby *et al.*, 1991), the north-south differentiation in *L. sealii* may have resulted by a process of isolation by distance where gene exchange occurs preferentially between neighbouring populations.

The return of adult fish to spawn in their natal rivers would also maintain separate genetic stocks however, this would also promote temporal stability of allele frequencies. *Lovettia sealii* from the Mersey River Mersey River collected over three successive spawning seasons showed marked genetic heterogeneity. Barker and Lambert (1988) suggested that for highly polymorphic loci, as illustrated by *PEPA*, high mortality is likey to result in differentiation of populations in different rivers, and this pattern would be expected to fluctuate from year to year.

A possible explanation for the in-site variabilty is the potential for high losses of both the spawning adults or larvae. The fecundity of *L. sealii* is extremely low with each female carrying between 150 and 300 eggs (Fulton *et al.*, 1985). McDowall (1968) in a major study of the species, found that fecundity of *G. maculatus* varied widely from 175 to 13,500 eggs. Parkinson (1984) observed significant differentiation in fecundity of *Salmo gairdneri* between populations in adjacent streams. In common with results observed in this study this species appears to be subdivided into a number of semi-isolated populations each having the potential to evolve adaptations to local environmental conditions. The differences observed between the Huon and Derwent Rivers indicates that the migration between sites is not great enough to result in genetic homogeneity. The genetic variation changed between spawning seasons. This may reflect that a mixed population was sampled within the Derwent River. It is possible that the *L. sealii* caught on different occasions was composed of varying proportions of Derwent and other southern river specimens. The genetic differentiation over distances smaller than the potential dispersal distance has been observed in a number of species (Parkinson, 1984). There are no data available of the dispersal capabilities of *L. sealii*. The limited distribution of this species can be used as evidence to support the existence of geographically restricted populations *L. sealii*. This species is limited to Tasmania, not even reaching the nearby Flinders and Cape Barren Islands (Fulton, 1990). McDowall explained the absence of *L. sealii* from the mainland Australia due to temperature limitations and an inability to compete with species in the more diverse fauna of mainland Australia. Despite the rapid movement of larvae downstream from spawning areas into estuaries there is no evidence that the larvae move any distance offshore. Despite extensive collecting efforts no *L. sealii* were collected from the east corner of Tasmania to the Tasman Peninsula. As a result it was not possible to examine if a transition zone exists between northern and southern Tasmania.

Southern Tasmanian rivers are also not a homogeneous genetic pool. The *PEP-A* locus is the key character used to define the population structure of *L. sealii*. In 1985 and 1987 the southern rivers appeared to sustain three genetic pools: Derwent, Parson's Bay Creek and South (all other southern rivers). In 1986 two key river populations (Derwent and Huon) appeared to be derived from a single genetic pool. Differential movement of larvae between these rivers could lead to the observed fluctuation in allele frequencies. The hydrological patterns in the south-east of Tasmania could also alternately isolate Derwent River larvae or move them to far southern rivers.

Data from the Catamaran, Lune and Huon rivers suggest that there is exchange of genetic material over southern Tasmania. When migration is very high, a species will mate randomly. If this species is split into arbitrarily delimited populations, the allele frequency should be the same, except for sampling variation, in all populations (Wehrhahn, 1987). The present study delimited the species by river. The stability of allele frequencies within but not between rivers over the spawning season suggest that the migration rate may be low. Conversely the variation observed within the Mersey River during 1985 may reflect genetic isolation within river systems. Larval *L. sealii* from northern rivers may drift downstream to the sea where they mix freely. There is no evidence of a homing instinct but it is possible that the adults return to their natal spawning grounds the following season. This would facilitate the genetic heterogeneity observed between adjacent northern rivers. It is more likey that larvae do not move out into Bass Strait and congregate as a single pool but, instead remain in their separate riverine schools close to the estuaries. Mersey spawned larvae returning as adults in winter. An influx of strays from other rivers may lead to the discordant results observed during a single season.

The broad separation of *L. sealii* into northern and southern stocks as proposed by Blackburn (1950) appears to be valid in the light of both historical catch data, morphometric analysis and the major discontinuity at the *PEP-A* locus between the north and south of Tasmania. However, an isolation by distance model (Wright, 1969) could explain the broad separation of this species into north and south genetic regions, where geographic distance is limiting the mixing of populations. The lack of hydrogeographic data for Tasmanian waters makes it difficult to predict the movement of larvae after hatching. It is therefore not possible to determine if larval mixing is prevented by the prevailing currents or that fish remain in isolated units in Bass Strait, the Tasman Sea or Indian Ocean. Tagging studies of this species are not possible and their observation at sea has only been anecdotal.

Population genetics studies the natural patterns of the preservation and evolution of the genotypic structure of populations in time and space. If there is a difference in timing between stocks, the economics of the fishery may concentrate the harvest on the earlier stock. Historically this was true of the northern *L. sealii* populations, where migration begins on average 1-2 months before fish in the south. The size and pigmentation of the southern fish also made them less desirable and therefore led to an increase in the fishing pressure upon northern populations. The north-south divide in this species may be the result of the population crash and subsequent genetic divergence which occurred as a result of fishing pressure in the north of the state earlier this century. It may also reflect long-term genetic divergence.

In southern Tasmania, there is no evidence of discrete multiple spawning runs in rivers. Genetic isolation would be expected if early and late season spawners did not interbreed within a river system. Cook Islet is characterised by two spawning runs of chinook salmon

(*Onchorhyncus tshawtscha*) in July and August in different areas of the drainage (Burger *et al.*, 1985). Gharrett *et al.* (1987) found that the composition of chinook stocks passing through the mouth of the Yukon River varied during the spawning run. The authors postulated that the size and remoteness of the tributaries of the Yukon indicated that it contains a number of genetic stocks. These are due to the variety of life histories (e.g. spawning near the river mouth or 160 km upstream) and restricted gene flow because of homing behaviour. Parkinson (1984) observed differences in repeat samples of steelhead populations from a single stream. The differences were thought to be due to the low density of spawning adults and limited juvenile dispersal. As a result the fish sampled at a single point may be the progeny of a limited number of parents and therefore have gene frequencies that differ from the mean of the population in the stream as a whole. This is unlikely to account for the variation in *L. sealii* observed in the Mersey River in 1985, as the variation occurs within repeat samples of spawning adults taken from a single area. The most likely explanation for this variation is the mis-scoring of loci by the investigator. The Mersey 1/85 sample was the first sample analysed in the study. Adults captured in the Mersey (1-3/85) and Rubicon Rivers (1/85) on the same day did not display any genetic heterogeneity. However *L. sealii* of the Inglis and Rubicon Rivers were genetically distinct on the basis of G-statistic results. This heterogeneity was present in northern Tasmanian rivers in 1986 where the Duck, Leven and Mersey Rivers all appeared to support their own stocks of this species.

Knowledge of the life history of *L. sealii* is incomplete. Eggs, larvae and mature adults have all been observed but the juvenile and its development to an adult has not been seen in the wild. The failure of spent *L. sealii* to rejuvinate is in keeping with some other salmonid species. The Japanese salmonoid, *Plecoglossus altivelis* Temminck and Schlegel has an annual life cycle, with most adults dying after breeding. *Salangichthys microdon* Bleeker also has a similar life history pattern (McDowall, 1978). The life cycles of the Pacific salmons (*Onchorhyncus spp.*) although including longer maturing life, end in almost total loss of the adult population after spawning. *Hypomesus pretiosus* is an osmerid in which the life cycle is annual and leads to high adult mortality (Loosanoff, 1937), although as in *G. maculatus* there are few 2 year-old maiden spawners. This life-history strategy has important implications for the potential genetic stability of a species. The selective removal of spawning adults leaves *L. sealii* very vulnerable to over-fishing. The fact that *L. sealii* sampled in this study are all of a uniform age means that genetic variability due to size and age was not a factor in the analysis. The only exception may be a very small proportion (0.1%) of 2-year old specimens which may occur in the population (Blackburn, 1950). These fish are generally characterised by their larger size and were excluded from the study samples.

Historically *L. sealii* has proven to be vunerable to overfishing. Current fisheries regulations introduced in Tasmania allow only recreational fishing for "whitebait". They serve to limit the amount of whitebait that can be removed from a river, the timing of the season and areas available for fishing. Blackburn in a major review of the *L. sealii* recommended a quota for the northern *L. sealii* catch for 1949 despite his report being published in 1950. However, the actual catch for this year did not approach the quota as the fishery was already in severe decline. The result was that there were not many fish left. Populations may be over-exploited by recruitment overfishing or by growth overfishing. In recruitment overfishing, a high mortality in all age classes ensures that few fish fish reach maturity and subsequent recruitment rates will be low. In growth overfishing, many individuals are caught when young. In *L. sealii* over-exploitation at any period in the life cycle will have profound effects on the numbers available for harvest the following season.

A similar situation can be seen in New Zealand where as early as 1928, the local whitebait fishery based primarly on *G. maculatus* was considered to be universally in decline (McDowall, 1968). This fishery dates from pre-European times and was rapidly developed at the end of the last century after the colonisation of New Zealand. By 1894, legislation was approved to regulate the whitebait fishery in New Zealand. In the North Island, where populations have been subjected to the highest fishing pressure, runs are sporadic and they vary from river to river and season to season. On the west coast of the South Island the fishery suffered a major decline in catch after 1955. There was some evidence of a recovery in the mid 1960s. It was suggested that the poor years were a result of the normal irregular fluctuation of the fishery which could not be explained. Both the Tasmanian *L. sealii* and New Zealand *G. maculatus* fisheries demonstrate natural variability and highlight the vulnerability of both species to overfishing. As well the construction of dams, river diversions, industrial and agricultural effuent can all profoundly effect the spawning habitat and the survival of larvae.

Roby *et al.* (1991) noted that among environmental factors known to exert a selective pressure on fish, temperature has been positively correlated with the genetic variability of a locus coding for malic enzyme and esterase loci. Roby *et al.* (1991) considered that local environmental factors may inflate the genetic divergence between samples and mask any concurrent high gene flow. There is no evidence to suggest that the variation observed at the peptidase locus is related to environmental selection. However, the allele frequencies at this locus are inconsistent with all others, Slatkin (1987) would suggest this reflects selection at that locus. The evolutionary significance of protein polymorphisms have been debated between "balance theory" advocates, who maintain that selection operates to preserve polymorphisms, and those that argue that genetic variation is neutral (DiMichele *et al.*, 1991). DiMichele *et al.* (1991) examined the effect of lactate dehydrogenase-B allozyme on the metabolism of the teleost, *Fundulus heteroclitus*. They concluded that allozyme variation is associated with metabolic differences that may affect fitness. In this way allozymic variation may be important in the evolution of species. Gauldie (1984) proposed that the use of allelic variation was inappropriate in establishing genetically isolated fish stocks. Instead the author considered that the variation represented an adaptive physiological response of a species to its environment. A study of the physiological implications of *ADA* or *PEP-A* allozymes observed in *G. maculatus* or *L. sealii* respectively is beyond the scope of this work. As a result it is not possible to conclude if the variation is due to chance, selective forces or a combination of both.

4.4 Management Implications

All biologically important characteristics of populations, including their size and productive efficiency, are determined by the historically established gene pools. Therefore, the population genetic analysis of species in nature is of primary importance in developing an optimal strategy for their efficient management (Altukhov, 1981).

Historically the mangement of Tasmanian "whitebait" has been by a single policy adopted for the whole State without regard for the different species involved and without any knowledge of their genetic structure. The "whitebait" were considered to be a single harvesting unit. From data collected from just two of the major "whitebait" species it is clear that this strategy is inappropriate. The present electrophoretic data would generally support the management of individual rivers, however this may not be feasible due to econommic and social pressures. Perkins *et al.* (1993) determined a minimum subset which should be maintained to preserve

genetic diversity in the brook trout of northeastern USA. This recommends that two representatives of each genetic stock are maintained and protected. For *L. sealii* this would mean that each of 2 northern and southern rivers are managed as separate units to reflect their unique genetic structure. The Derwent River would form a third mangement unit.

4.5 Conclusions

This study has demonstated that *L. sealii* exhibit significant genetic heterogeneity throughout the known species range. Similarly *G. maculatus* exists as a number of unique stocks. The fishing regulations and management of *L. sealii* should focus on the protection of individual stocks and not geographic regions. The present study has provided preliminary information about the population structure of this species however, more extensive sampling within Tasmania particularly an effort to obtain samples from the east and south-west coasts would be most informative. To date it is only possible to speculate on the geographic boundaries between stocks. The situation appears to be fluid and the stability of these boundaries unpredictable.

For both species several issues remain to be resolved: Where do larvae go once they hatch? Do the larvae simply drift upon ocean currents? What part of the water column do they occupy while at sea? Do they school? Why does the species have such a restricted range or does this reflect the limited areas that have been surveyed ? If not, what is the barrier to further dispersal in this species? The absence of life history data of both the larval stage of *G. maculatus* and the void in knowledge surrounding *L. sealii* between the time the larvae drift downstream to their return as mature adults make decisions regarding their management necessarily conservative.

Appendix 2a: Sample Preparation Buffer

NADP	10mg
ß-Mercaptoethanol	100μl
Triton-X 100	100μl
Distilled water	100ml

Appendix 2b: Electrophoresis Running Buffers

1. (0.01M) Citrate-Phosphate pH6.4

   (10mM) Na2HPO4.12H2O 3.58g
   (2.5mM) Citric acid 0.53g
   in 1 litre of distilled water.

2. (0.05M) Tris-Maleate pH7.8

   (50mM) Tris 6.06g
   (20mM) Maleic acid 2.32g
   in 1 litre of distilled water.

3. (0.02M) Phosphate pH7.0

   (11.6mM) Na2HPO4.12H2O 4.15g
   (8.4mM) NaH2PO4.2H2O 1.31g
   in 1 litre of distilled water.

Appendix 2c: Enzyme Histochemical Staining Recipes and Optimal Running Conditions.

1. Peptidase (PEP) EC 3.4.11

O-Dianisidine diHCl	8 mg
0.1M Tris-HCl pH 8.0	2 ml
0.2M $MgCl_2$	0.1 ml
L-amino acid oxidase	2 mg
Peroxidase	2 mg
Substrates:	
Peptidase A leucine-glycine	10 mg
Peptidase D phenylalanine-proline	10 mg

Peptidase A and D were examined in extracts of muscle tissue and migrates anodally in (0.01M) Citrate-phosphate buffer pH 6.4. at 200V for $1^3/4$ hr at 4°C.

2. Phosphogluconate Dehydrogenase (PGD) **EC 1.1.1.44**

6-phosphogluconic acid	5 mg
0.1M Tris-HCl pH 8.0	2 ml
NADP	0.1 ml
1M $MgCl_2$	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

Phoshogluconate dehydrogenase was examined in extracts of muscle tissue and migrates anodally in (0.02M) Phosphate buffer pH 7.0 at 200V for 2 hrs at 4°C.

3. Adenosine Deaminase (ADA) **EC 3.5.4.4**

adenosine	10 mg
Tris-HCl pH 8.0	2 ml
Arsenate	0.1 ml
MTT	0.1 ml
PMS	0.1 ml
nucleoside phosphorylase	1 I.U.
xanthine oxidase	1 I.U.

Adenosine deaminase was examined in extracts of muscle tissue and migrates anodally in (0.02M) Phosphate buffer pH 7.0. Gels are run at 200V for 1 3/4 hrs at 200v at 4°C.

4. Phosphoglucomutase (PGM) **EC 5.4.2.2**

glucose-1-phosphate	10 mg
D-glucose-1-6-diphosphate	0.2 mg
0.1M Tris-HCl pH 8.0	2 ml
NADP	0.1 ml
1M $MgCl_2$	0.1 ml
MTT	0.1 ml
PMS	0.1 ml
glucose-6-phosphate dehydrogenase	2 I.U.

Phosphoglucomutase was examined in muscle tissue extracts and migrates anodally in 0.05M Tris-Maleate buffer pH 7.8 at 200V for 2 hrs at 4°C.

5. Glucose Phosphate Isomerase (GPI2) **EC 5.3.1.9**

fructose-6-phosphate	5 mg
0.1M Tris-HCl	2 ml
NADP	0.1 ml
1M $MgCl_2$	0.1 ml
MTT	0.1 ml
PMS	0.1 ml
glucose-6-phosphate dehydrogenase	2 I.U.

Glucose phosphate isomerase was examined in muscle tissue extracts and migrates anodally in 0.05M Tris-Maleate pH 7.8 at 200V for 2 hrs at4°C.

6. Glutamate Pyruvate Transaminase (GPT) EC 2.6.1.2

L-alanine	10 mg
NADH	3 mg
0.1M Tris-HCL	2 ml
a-ketoglutarate	0.2 ml
lactate dehydrogenase	2 I.U.

View under uv light, counter-stain with 0.2 ml MTT/PMS in 2 ml water.
Glutamate pyruvate transaminase was examined in muscle extracts and migrates anodally in 0.01M Citrate-Phosphate buffer pH 6.4 at 200V for 1 3/4 hrs at 4°C.

7. Mannose phosphate dehydrogenase (MPI) E.C. 5.3.1.8

mannose-6-phosphate	8 mg
0.1M Tris-HCl pH 8.0	2 ml
NADP	0.1 ml
1M $MgCl_2$	0.1 ml
MTT	0.1 ml
PMS	0.1 ml
glucose phosphate isomerase	2 I.U.
glucose-6-phosphate dehydrogenase	2 I.U.

Mannose phosphate isomerase was examined in muscle tissue extracts and migrates anodally in 0.02M Phosphate buffer pH 7.0 at 200V for 2.5 hrs at 4°C.

8. Lactate dehydrogenase (LDH) E.C. 1.1.1.27

0.1M Tris-Hcl pH 8.0	2 ml
lactate	0.1 ml
NAD	0.1 ml
MTT	0.1 ml
PMS	0.1 ml

Lactate dehydrogenase was examined in muscle tissue extracts and migrates anodally in 0.02M Phosphate buffer pH 7.0 at 200v for 2.5 hours at 4°C.

9. Glycerol-3-phosphate dehydrogenase (aGPD) E.C.1.1.1.8

L-glycerol-3-phosphate	10 mg
0.1M Tris-HCl pH 8.0	2 ml
NAD	0.1 ml
MTT	0.1 ml
PMS	0.1 ml
pyruvate	0.1 ml

Glycerol phosphate dehydrogenase was examined in muscle tissue extracts and migrates anodally in 0.02M Phosphate buffer pH 7.0 at 200v for 1.5 hours.

10. Aspartate amino transferase (GOT) E.C. 2.6.1.1

Fast garnet GBC salt	6 mg
0.1M Tris-HCl pH 8.0	2 ml
a-ketoglutarate 50 mg/ml	0.2 ml
L-aspartate 50 mg/ml	0.2 ml

Aspartate amino transferase was examined in muscle tissue extracts and migrates anodally in 0.02M Phosphate buffer at 200v for 1.5 hours.

Appendix 2d: Stock solutions used in enzyme-specific stain recipes

Solution	Concentration
MgCl2 (0.2M)	40.6mg/ml
MgCl2 (1M)	203mg/ml
NADP	20mg/ml
MTT	6mg/ml
PMS	2mg/ml
Arsenate	6mg/ml
a-Ketoglutarate	50mg/ml (pH 8)
Lactate	50mg/ml (pH8)
Pyruvate	50mg/ml

Appendix 3a: *L. Sealii* Chi-square test with pooling

Population: MERSEY1/85 (LS1)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
LDH2	Homozygotes for most common allele	65	68.134			
	Common/rare heterozygotes	36	29.731			
	Rare homozygotes and other heterozygotes	0	3.134	4.600	1	.032

Significance test using exact probabilities

****

Population: MERSEY1/85 (LS1)

Locus	R1	R2	R3	P
GPD1	76	18	4	.060
G6PD	99	1	0	1.000
PEPA	89	6	1	.141
6PGD	94	7	1	.168
LDH2	65	36	0	.037
GOT2	50	36	15	.069

Chi-square test with pooling

****

Population: MERSEY2/85 (LS2)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	85	84.467			
	Common/rare heterozygotes	12	13.067			
	Rare homozygotes and other heterozygotes	1	.467	.700	1	.403
GOT2	Homozygotes for most common allele	34	35.041			
	Common/rare heterozygotes	50	47.919			
	Rare homozygotes and other heterozygotes	15	16.041	.189	1	.664

Significance test using exact probabilities

****

Population: MERSEY2/85 (LS2)

Locus	R1	R2	R3	P
GPD1	86	13	1	.433
G6PD	89	11	0	1.000
PEPA	85	12	1	.393
6PGD	90	7	3	.003
LDH2	42	35	3	.250
GOT2	34	50	15	.682

Chi-square test with pooling

****

Population: MERSEY3/85 (LS3)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	83	82.769			
	Common/rare heterozygotes	16	16.462			
	Rare homozygotes and other heterozygotes	1	.769	.083	1	.773
6PGD	Homozygotes for most common allele	79	79.161			
	Common/rare heterozygotes	20	19.678			
	Rare homozygotes and other heterozygotes	1	1.161	.028	1	.867
GOT2	Homozygotes for most common allele	40	37.684			
	Common/rare heterozygotes	36	40.633			
	Rare homozygotes and other heterozygotes	13	10.684	1.173	1	.279

Significance test using exact probabilities

****

Population: MERSEY3/85 (LS3)

Locus	R1	R2	R3	P
GPD1	95	5	0	1.000
G6PD	94	6	0	1.000
PEPA	83	16	1	.568
6PGD	79	20	1	1.000
LDH2	54	37	1	.064
GOT2	40	36	13	.350
MPI	99	1	0	1.000

Chi-square test with pooling

****

Population: MERSEY5/85 (LS4)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	81	80.955			
	Common/rare heterozygotes	18	18.090			
	Rare homozygotes and other heterozygotes	1	.955	.003	1	.959
LDH2	Homozygotes for most common allele	52	56.156			
	Common/rare heterozygotes	46	37.688			
	Rare homozygotes and other heterozygotes	2	6.156	4.946	1	.026

Significance test using exact probabilities

****

Population: MERSEY5/85 (LS4)

Locus	R1	R2	R3	P
GPD1	90	9	1	.252
G6PD	96	4	0	1.000
PEPA	81	18	1	1.000
6PGD	73	26	1	.685
LDH2	52	46	2	.031
GOT2	37	45	16	.678

Significance test using exact probabilities

****

Population: RUB1/85 (LS5)

Locus	R1	R2	R3	P
GPD1	77	16	3	.100
G6PD	95	1	0	1.000
PEPA	89	6	1	.141
6PGD	72	23	1	1.000
LDH2	56	34	6	.781
GOT2	41	38	17	.131

Chi-square test with pooling

****

Population: INGLIS85 (LS6)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
6PGD	Homozygotes for					

most common allele	59	60.720			
Common/rare heterozygotes	34	30.561			
Rare homozygotes and other heterozygotes	2	3.720	1.231	1	.267

Significance test using exact probabilities

****

Population: INGLIS85 (LS6)

Locus	R1	R2	R3	P
GPD1	89	6	0	1.000
G6PD	90	5	0	1.000
PEPA	76	16	3	.102
6PGD	59	34	2	.347
LDH2	38	40	1	.009
GOT2	35	46	16	1.000

Chi-square test with pooling

****

Population: ALLENS85 (LS7)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPD1	Homozygotes for most common allele	92	92.107			
	Common/rare heterozygotes	7	6.787			
	Rare homozygotes and other heterozygotes	0	.107	.113	1	.736
G6PD	Homozygotes for most common allele	91	91.181			
	Common/rare heterozygotes	9	8.638			
	Rare homozygotes and other heterozygotes	0	.181	.196	1	.658
PEPA	Homozygotes for most common allele	34	30.126			
	Common/rare heterozygotes	42	49.749			
	Rare homozygotes and other heterozygotes	24	20.126	2.451	1	.117
6PGD	Homozygotes for most common allele	74	75.633			
	Common/rare heterozygotes	26	22.734			
	Rare homozygotes and other heterozygotes	0	1.633	2.138	1	.144
GOT2	Homozygotes for					

	most common allele	42	45.452			
	Common/rare heterozygotes	51	44.095			
	Rare homozygotes and other heterozygotes	7	10.452	2.484	1	.115
MPI	Homozygotes for most common allele	97	97.030			
	Common/rare heterozygotes	4	3.940			
	Rare homozygotes and other heterozygotes	0	.030	.031	1	.861

Significance test using exact probabilities

****

Population: ALLENS85 (LS7)

Locus	R1	R2	R3	P
GPD1	92	7	0	1.000
G6PD	91	9	0	1.000
PEPA	34	42	24	.156
6PGD	74	26	0	.362
LDH2	59	38	2	.227
GOT2	42	51	7	.169
MPI	97	4	0	1.000

Chi-square test with pooling

****

Population: DWT85 (LS8)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
G6PD	Homozygotes for most common allele	74	73.273			
	Common/rare heterozygotes	8	9.455			
	Rare homozygotes and other heterozygotes	1	.273	2.170	1	.141
PEPA	Homozygotes for most common allele	33	30.130			
	Common/rare heterozygotes	33	38.739			
	Rare homozygotes and other heterozygotes	15	12.130	1.802	1	.179

6PGD Homozygotes for most common allele	69	68.305			
Common/rare heterozygotes	18	19.390			
Rare homozygotes and other heterozygotes	2	1.305	.477	1	.490
GOT2 Homozygotes for most common allele	43	39.446			
Common/rare heterozygotes	32	39.109			
Rare homozygotes and other heterozygotes	13	9.446	2.950	1	.086

Significance test using exact probabilities

****

Population: DWT85 (LS8)

Locus	R1	R2	R3	P
G6PD	74	8	1	.250
PEPA	33	33	15	.241
6PGD	69	18	2	.614
LDH2	57	28	4	.748
GOT2	43	32	13	.096
MPI	87	2	0	1.000
GOT1	99	1	0	1.000

Chi-square test with pooling

****

Population: PARSON'S85 (LS9)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA Homozygotes for most common allele		9	11.148			
Common/rare heterozygotes		25	20.704			
Rare homozygotes and other heterozygotes		7	9.148	1.810	1	.179
GOT2 Homozygotes for most common allele		16	13.684			
Common/rare heterozygotes		15	19.633			
Rare homozygotes and other heterozygotes		9	6.684	2.288	1	.130

Significance test using exact probabilities

****

Population: PARSON'S85 (LS9)

Locus	R1	R2	R3	P
GPD1	39	1	0	1.000
PEPA	9	25	7	.218
6PGD	39	1	0	1.000
LDH2	22	11	1	1.000
GOT2	16	15	9	.191

Chi-square test with pooling

****

Population: LUNE85 (LS10)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	38	34.921			
	Common/rare heterozygotes	40	46.157			
	Rare homozygotes and other heterozygotes	18	14.921	1.728	1	.189

LUNE 85 cont.

GOT2	Homozygotes for most common allele	56	54.660			
	Common/rare heterozygotes	33	35.681			
	Rare homozygotes and other heterozygotes	7	5.660	.552	1	.458
MPI	Homozygotes for most common allele	92	92.031			
	Common/rare heterozygotes	4	3.937			
	Rare homozygotes and other heterozygotes	0	.031	.032	1	.857

Significance test using exact probabilities

****

Population: LUNE85 (LS10)

Locus	R1	R2	R3	P
GPD1	94	1	0	1.000
G6PD	90	6	0	1.000
PEPA	38	40	18	.206
6PGD	78	14	3	.060
LDH2	54	37	4	.577
GOT2	56	33	7	.579
MPI	92	4	0	1.000

Chi-square test with pooling

****
Population: HUON1/85 (LS11)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
6PGD	Homozygotes for most common allele	87	86.457			
	Common/rare heterozygotes	12	13.085			
	Rare homozygotes and other heterozygotes	1	.457	.738	1	.390
LDH2	Homozygotes for most common allele	53	53.925			
	Common/rare heterozygotes	41	39.151			
	Rare homozygotes and other heterozygotes	6	6.925	.227	1	.634
GOT2	Homozygotes for most common allele	51	50.307			
	Common/rare heterozygotes	40	41.387			
	Rare homozygotes and other heterozygotes	9	8.307	.114	1	.736

Significance test using exact probabilities
****
Population: HUON1/85 (LS11)

Locus	R1	R2	R3	P
G6PD	88	12	0	1.000
PEPA	45	47	8	.488
6PGD	87	12	1	.386
LDH2	53	41	6	.798
GOT2	51	40	9	.809

Chi-square test with pooling
****
Population: HUON2/85 (LS12)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	48	43.667			
	Common/rare heterozygotes	35	43.667			
	Rare homozygotes and other heterozygotes	15	10.667	3.911	1	.048

6PGD Homozygotes for most common allele	75	74.538			
Common/rare heterozygotes	21	21.923			
Rare homozygotes and other heterozygotes	2	1.538	.180	1	.671
GOT2 Homozygotes for most common allele	40	42.563			
Common/rare heterozygotes	50	44.873			
Rare homozygotes and other heterozygotes	9	11.563	1.308	1	.253
MPI Homozygotes for most common allele	94	93.031			
Common/rare heterozygotes	2	3.938			
Rare homozygotes and other heterozygotes	1	.031	31.161	1	.000

Significance test using exact probabilities

****

Population: HUON2/85 (LS12)

Locus	R1	R2	R3	P
G6PD	87	10	1	.301
PEPA	48	35	15	.067
6PGD	75	21	2	.650
LDH2	47	48	1	.003
GOT2	40	50	9	.273
MPI	94	2	1	.031
GOT1	96	3	0	1.000

Chi-square test with pooling

****

Population: MERSEY (LS13)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPD1 Homozygotes for most common allele		89	89.276			
Common/rare heterozygotes		11	10.447			
Rare homozygotes and other heterozygotes		0	.276	.306	1	.580
PEPA Homozygotes for most common allele		79	76.508			
Common/rare heterozygotes		17	21.985			
Rare homozygotes and other heterozygotes		4	1.508	5.332	1	.021

GOT2 Homozygotes for most common allele	47	50.385			
Common/rare heterozygotes	37	30.231			
Rare homozygotes and other heterozygotes	1	4.385	4.356	1	.037

Significance test using exact probabilities

****

Population: MERSEY/86 (LS13)

Locus	R1	R2	R3	P
GPD1	89	11	0	1.000
G6PD	90	6	0	1.000
PEPA	79	17	4	.042
6PGD	83	17	0	1.000
LDH2	54	36	8	.605
GOT2	47	37	1	.060

Chi-square test with pooling

****

Population: MERSEY4/86 (LS14)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPD1	Homozygotes for most common allele	92	92.141			
	Common/rare heterozygotes	8	7.719			
	Rare homozygotes and other heterozygotes	0	.141	.151	1	.697
PEPA	Homozygotes for most common allele	78	79.161			
	Common/rare heterozygotes	22	19.678			
	Rare homozygotes and other heterozygotes	0	1.161	1.452	1	.228
GOT2	Homozygotes for most common allele	32	37.090			
	Common/rare heterozygotes	58	47.819			
	Rare homozygotes and other heterozygotes	10	15.090	4.583	1	.032

Significance test using exact probabilities

****

Population: MERSEY4/86 (LS14)

Locus	R1	R2	R3	P

GPD1	92	8	0	1.000
G6PD	97	3	0	1.000
PEPA	78	22	0	.600
6PGD	60	24	4	.470
LDH2	55	40	4	.419
GOT2	32	58	10	.037

Chi-square test with pooling

****

Population: DUCK86 (LS15)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	74	75.523			
	Common/rare heterozygotes	25	21.954			
	Rare homozygotes and other heterozygotes	0	1.523	1.976	1	.160
6PGD	Homozygotes for most common allele	71	72.061			
	Common/rare heterozygotes	27	24.878			
	Rare homozygotes and other heterozygotes	1	2.061	.743	1	.389
GOT2	Homozygotes for most common allele	44	43.447			
	Common/rare heterozygotes	44	45.106			
	Rare homozygotes and other heterozygotes	12	11.447	.061	1	.805

Significance test using exact probabilities

****

Population: DUCK86 (LS15)

Locus	R1	R2	R3	P
G6PD	96	4	0	1.000
PEPA	74	25	0	.354
6PGD	71	27	1	.686
LDH2	55	40	4	.419
GOT2	44	44	12	.826
MPI	95	5	0	1.000

Chi-square test with pooling

****

Population: LEVEN86 (LS16)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
G6PD	Homozygotes for most common allele	90	90.226			
	Common/rare heterozygotes	10	9.548			
	Rare homozygotes and other heterozygotes	0	.226	.248	1	.618
GOT2	Homozygotes for most common allele	37	35.281			
	Common/rare heterozygotes	45	48.437			
	Rare homozygotes and other heterozygotes	18	16.281	.509	1	.476

Significance test using exact probabilities

****

Population: LEVEN86 (LS16)

Locus	R1	R2	R3	P
GPD1	81	16	3	.090
G6PD	90	10	0	1.000
PEPA	95	4	1	.074
6PGD	75	13	7	.000
LDH2	72	25	3	.699
GOT2	37	45	18	.535

Significance test using exact probabilities

****

Population: CAT86 (LS17)

Locus	R1	R2	R3	P
G6PD	34	2	0	1.000
PEPA	11	20	4	.479
6PGD	21	7	1	.518
LDH2	24	10	1	1.000
GOT2	21	12	3	.653

Chi-square test with pooling

****

Population: DWT1/86 (LS18)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	32	28.200			
	Common/rare heterozygotes	30	37.600			
	Rare homozygotes and other heterozygotes	16	12.200	3.232	1	.072
6PGD	Homozygotes for most common allele	52	52.889			
	Common/rare heterozygotes	16	14.222			
	Rare homozygotes and other heterozygotes	0	.889	1.126	1	.289
LDH2	Homozygotes for most common allele	46	46.603			
	Common/rare heterozygotes	19	17.794			
	Rare homozygotes and other heterozygotes	1	1.603	.316	1	.574
GOT2	Homozygotes for most common allele	30	27.901			
	Common/rare heterozygotes	26	30.198			
	Rare homozygotes and other heterozygotes	10	7.901	1.299	1	.254

Significance test using exact probabilities

****

Population: DWT1/86 (LS18)

Locus	R1	R2	R3	P
G6PD	67	1	0	1.000
PEPA	32	30	16	.097
6PGD	52	16	0	.583
LDH2	46	19	1	1.000
GOT2	30	26	10	.285
GOT1	62	6	0	1.000

Chi-square test with pooling

****

Population: DWT2/86 (LS19)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
G6PD	Homozygotes for most common allele	87	87.188			
	Common/rare heterozygotes	9	8.623			
	Rare homozygotes and other heterozygotes	0	.188	.205	1	.650
PEPA	Homozygotes for most common allele	28	27.467			
	Common/rare heterozygotes	48	49.067			
	Rare homozygotes and other heterozygotes	22	21.467	.047	1	.829
6PGD	Homozygotes for most common allele	73	74.325			
	Common/rare heterozygotes	23	20.351			
	Rare homozygotes and other heterozygotes	0	1.325	1.693	1	.193
GOT2	Homozygotes for most common allele	49	48.775			
	Common/rare heterozygotes	39	39.450			
	Rare homozygotes and other heterozygotes	8	7.775	.013	1	.910

Significance test using exact probabilities

****

Population: DWT2/86 (LS19)

Locus	R1	R2	R3	P
G6PD	87	9	0	1.000
PEPA	28	48	22	.842
6PGD	73	23	0	.350
LDH2	46	15	3	.348
GOT2	49	39	8	1.000

Chi-square test with pooling

****

Population: Huon86 (LS20)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPD1	Homozygotes for most common allele	96	96.030			
	Common/rare heterozygotes	4	3.940			
	Rare homozygotes and other heterozygotes	0	.030	.031	1	.860
PEPA	Homozygotes for most common allele	44	42.136			
	Common/rare heterozygotes	42	45.729			
	Rare homozygotes and other heterozygotes	14	12.136	.673	1	.412
6PGD	Homozygotes for most common allele	73	73.899			
	Common/rare heterozygotes	26	24.201			
	Rare homozygotes and other heterozygotes	1	1.899	.571	1	.450
GOT2	Homozygotes for most common allele	40	43.447			
	Common/rare heterozygotes	52	45.106			
	Rare homozygotes and other heterozygotes	8	11.447	2.365	1	.124

Significance test using exact probabilities

****

Population: HUON86 (LS20)

Locus	R1	R2	R3	P
GPD1	96	4	0	1.000
G6PD	93	7	0	1.000
PEPA	44	42	14	.509
6PGD	73	26	1	.685
LDH2	71	22	7	.016
GOT2	40	52	8	.179
GOT1	97	3	0	1.000

Chi-square test with pooling

****

Population: HUON86 (LS21)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P

GPD1 Homozygotes for most common allele	96	96.030			
Common/rare heterozygotes	4	3.940			
Rare homozygotes and other heterozygotes	0	.030	.031	1	.860
PEPA Homozygotes for most common allele	44	42.363			
Common/rare heterozygotes	43	46.274			
Rare homozygotes and other heterozygotes	14	12.363	.512	1	.474
6PGD Homozygotes for most common allele	73	73.899			
Common/rare heterozygotes	26	24.201			
Rare homozygotes and other heterozygotes	1	1.899	.571	1	.450
GOT2 Homozygotes for most common allele	40	43.447			
Common/rare heterozygotes	52	45.106			
Rare homozygotes and other heterozygotes	8	11.447	2.365	1	.124

Significance test using exact probabilities

****

Population: HUON86 (LS21)

Locus	R1	R2	R3	P
GPD1	96	4	0	1.000
G6PD	93	7	0	1.000
PEPA	44	43	14	.516
6PGD	73	26	1	.685
LDH2	71	22	7	.016
GOT2	40	52	8	.179
GOT1	97	3	0	1.000

Chi-square test with pooling

****

Population: CAT87 (LS22)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
G6PD	Homozygotes for most common allele	83	82.769			

Common/rare heterozygotes	16	16.462			
Rare homozygotes and other heterozygotes	1	.769	.083	1	.773
PEPA Homozygotes for most common allele	37	39.573			
Common/rare heterozygotes	52	46.854			
Rare homozygotes and other heterozygotes	11	13.573	1.220	1	.269
GOT2 Homozygotes for most common allele	48	51.739			
Common/rare heterozygotes	48	40.523			
Rare homozygotes and other heterozygotes	4	7.739	3.456	1	.063

Significance test using exact probabilities

****

Population: CAT87 (LS22)

Locus	R1	R2	R3	P
GPD1	98	2	0	1.000
G6PD	83	16	1	.568
PEPA	37	52	11	.291
6PGD	75	22	3	.399
LDH2	69	28	3	1.000
GOT2	48	48	4	.082
GOT1	99	1	0	1.000

Chi-square test with pooling

****

Population: PARSON'S87 (LS23)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	28	29.330			
	Common/rare heterozygotes	52	49.340			
	Rare homozygotes and other heterozygotes	19	20.330	.291	1	.590

GOT2 Homozygotes for most common allele	53	49.598			
Common/rare heterozygotes	35	41.804			
Rare homozygotes and other heterozygotes	12	8.598	2.687	1	.101

Significance test using exact probabilities

****

Population: PARSON'S87 (LS23)

Locus	R1	R2	R3	P
G6PD	98	2	0	1.000
PEPA	28	52	19	.685
6PGD	94	6	0	1.000
LDH2	63	33	4	1.000
GOT2	53	35	12	.146
GOT1	97	3	0	1.000

Chi-square test with pooling

****

Population: DWT87 (LS24)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
6PGD	Homozygotes for most common allele	81	80.955			
	Common/rare heterozygotes	18	18.090			
	Rare homozygotes and other heterozygotes	1	.955	.003	1	.959

Significance test using exact probabilities

****

Population: DWT87 (LS24)

Locus	R1	R2	R3	P
GPD1	94	1	0	1.000
G6PD	88	12	0	1.000
PEPA	23	44	21	1.000
6PGD	81	18	1	1.000
LDH2	58	29	3	1.000
GOT2	46	41	8	1.000

Chi-square test with pooling

****

Population: NWBAY87 (LS25)

		Observed	Expected	Chi-

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
G6PD	Homozygotes for most common allele	28	27.159			
	Common/rare heterozygotes	3	4.683			
	Rare homozygotes and other heterozygotes	1	.159	5.089	1	.024
PEPA	Homozygotes for most common allele	13	14.333			
	Common/rare heterozygotes	17	14.333			
	Rare homozygotes and other heterozygotes	2	3.333	1.153	1	.283
LDH2	Homozygotes for most common allele	21	19.444			
	Common/rare heterozygotes	8	11.111			
	Rare homozygotes and other heterozygotes	3	1.444	2.671	1	.102

Significance test using exact probabilities

****

Population: NWBAY87 (LS25)

Locus	R1	R2	R3	P
G6PD	28	3	1	.151
PEPA	13	17	2	.425
6PGD	25	6	1	.392
LDH2	21	8	3	.133
GOT2	20	9	3	.313

Chi-square test with pooling

****

Population: PIEMAN87 (LS26)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
GPD1	Homozygotes for most common allele	93	92.141			
	Common/rare heterozygotes	6	7.719			
	Rare homozygotes and other heterozygotes	1	.141	5.639	1	.018
PEPA	Homozygotes for most common allele	61	60.084			
	Common/rare heterozygotes	30	31.832			
	Rare homozygotes and other heterozygotes	5	4.084	.325	1	.569
GOT2	Homozygotes for					

most common allele	47	45.914			
Common/rare heterozygotes	41	43.173			
Rare homozygotes and other heterozygotes	11	9.914	.254	1	.614

Significance test using exact probabilities

****

Population: PIEMAN87 (LS26)

Locus	R1	R2	R3	P
GPD1	93	6	1	.135
G6PD	98	2	0	1.000
PEPA	61	30	5	.547
6PGD	73	23	4	.244
LDH2	76	21	3	.378
GOT2	47	41	11	.647

Chi-square test with pooling

****

Population: MERSEY87 (LS27)

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
PEPA	Homozygotes for most common allele	84	84.296			
	Common/rare heterozygotes	22	21.408			
	Rare homozygotes and other heterozygotes	1	1.296	.085	1	.771
6PGD	Homozygotes for most common allele	78	78.478			
	Common/rare heterozygotes	23	22.044			
	Rare homozygotes and other heterozygotes	1	1.478	.199	1	.656

Significance test using exact probabilities

****

Population: MERSEY87 (LS27)

Locus	R1	R2	R3	P
GPD1	90	11	0	1.000
G6PD	99	1	0	1.000
PEPA	84	22	1	1.000
6PGD	78	23	1	1.000
LDH2	56	41	3	.263
GOT2	50	36	14	.106
MPI	99	1	0	1.000
GOT1	99	1	0	1.000

Appendix 3b: F-Statistics (FIS(IK) values) for individual loci

Locus: *GPD1*

Allele	Subpopulation 1	2	3	4	5	6	7	8	9
A	.202	.063	-.026	.134	.179	-.033	-.031	...	-.013
B	.202	.063	-.026	.134	.179	-.033	-.037	...	-.013
C	...	...	...	...	...	...	-.005	...	...
D	...	...	...	...	...	...	...	...	...
Mean	.202	.063	-.026	.134	.179	-.033	-.032	...	-.013

Allele	Subpopulation 10	11	12	13	14	15	16	17	18
A	-.005	...	...	-.053	-.036	...	.183	...	...
B	-.005	...	...	-.058	-.042	...	.183	...	...
C	...	...	...	-.005	-.005	...	...	...	...
D	...	...	...	...	...	...	...	...	...
Mean	-.005	...	...	-.053	-.037	...	.183	...	...

Allele	Subpopulation 19	20	21	22	23	24	25	26	27
A	...	-.010	-.010	-.010	...	...	...	-.020	-.058
B	...	-.020	-.020	-.010	...	-.005	...	.219	-.058
C	...	-.010	-.010	...	...	...	...	-.020	...
D	...	...	...	...	...	-.005	...	...	...
Mean	...	-.015	-.015	-.010	...	-.005	...	.098	-.058

Allele	F(IS)	F(IT)	F(ST)
A	.084	.131	.051
B	.087	.130	.047
C	-.012	-.002	.010
D	-.005	-.000	.005
Mean	.082	.126	.048

FIS(IK) values

****

Locus: *G6PD*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	-.005	-.058	-.031	-.020	-.005	-.027	-.042	.178	...
B	-.005	-.058	-.031	-.020	-.005	-.027	-.047	.149	...
C	...	...	...	...	...	...	-.005	-.006	...
Mean	-.005	-.058	-.031	-.020	-.005	-.027	-.042	.154	...

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	-.032	-.064	.112	-.032	-.015	-.020	-.026	-.029	-.007
B	-.032	-.064	.112	-.032	-.015	-.020	-.053	-.029	-.007
C	...	...	...	...	...	...	-.026	...	...
Mean	-.032	-.064	.112	-.032	-.015	-.020	-.039	-.029	-.007

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	-.043	-.036	-.036	.095	-.010	-.064	-.032	-.010	-.005
B	-.049	-.036	-.036	.023	-.010	-.064	.349	-.010	-.005
C	-.005	...	...	-.026	...	...	-.049	...	...
Mean	-.044	-.036	-.036	.042	-.010	-.064	.150	-.010	-.005

Allele	F(IS)	F(IT)	F(ST)
A	-.003	.010	.013
B	.019	.036	.017
C	-.032	-.004	.027
Mean	.006	.022	.016

FIS(IK) values

****

Locus: *PEPA*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	...	...	...	...	...	...	...
B	...	-.005	-.010	...	...	...	...	-.013	-.012
C	.217	.112	.134	.158	.217	.177	.152	.100	-.177
D	.217	.077	.023	.000	.217	.177	.129	.143	-.222
E	...	-.005	-.026	-.053	...	...	-.005	-.006	...
Mean	.217	.086	.048	.027	.217	.177	.139	.117	-.195

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	...	...	...	...	...	...	...	...	...
B	-.005	...	-.037	.113	-.015	-.031	...	...	...
C	.129	-.089	.194	.579	-.036	-.053	.313	-.190	.197
D	.156	-.089	.332	.223	-.124	-.145	.313	-.190	.165
E	-.016	...	-.005	-.015	-.064	-.048	...	...	-.006
Mean	.136	-.089	.236	.255	-.085	-.093	.313	-.190	.179

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	...	...	...	1.000	...	...	...	...
B	-.005	-.005	-.005	-.005	-.021	...	-.032	-.011	...
C	.017	.077	.066	-.115	.008	-.001	-.205	.053	-.115
D	-.005	.049	.054	-.114	-.059	-.001	-.123	.081	-.032
E	...	...	-.005	-.010	...	...	...	...	-.009
Mean	.006	.062	.058	-.111	-.006	-.001	-.156	.064	-.069

Allele	F(IS)	F(IT)	F(ST)
A	1.000	1.000	.010
B	.007	.028	.020
C	.030	.339	.319
D	.023	.312	.296
E	-.038	-.010	.027
Mean	.025	.314	.296

FIS(IK) values

****

Locus: *6PGD*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	...	...	...	-.005	...	...	...
C	.186	.424	-.021	-.080	-.058	-.118	-.149	.066	-.013
D	.186	.424	-.011	-.080	-.058	-.086	-.143	-.127	-.013
E	...	...	-.005	...	...	-.011	-.005	-.011	...
Mean	.186	.424	-.016	-.080	-.058	-.098	-.143	-.025	-.013

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	...	...	...	...	...	...	...	...	...
C	.218	.078	.037	-.093	.083	-.091	.439	.079	-.133
D	.218	.095	.050	-.093	.083	-.063	.439	.079	-.115
E	...	-.005	-.005	...	...	-.015	...	...	-.015
Mean	.218	.083	.043	-.093	.083	-.074	.439	.079	-.117

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	...	...	...	...	...	...	...	...
C	-.136	-.080	-.080	.086	-.031	.000	.143	.122	-.048
D	-.129	-.061	-.061	.086	-.031	.036	.143	.122	-.039
E	-.005	-.010	-.010	...	...	-.015	...	...	-.005
Mean	-.130	-.068	-.068	.086	-.031	.014	.143	.122	-.043

Allele	F(IS)	F(IT)	F(ST)
A	-.005	-.000	.005
C	.023	.040	.017
D	.027	.043	.017
E	-.011	-.004	.006
Mean	.024	.040	.017

FIS(IK) values
****
Locus: *LDH2*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	...	...	...	...	...	...	...
B	-.202	-.148	-.204	-.216	.028	-.297	-.148	.025	-.046
C	-.217	-.148	-.204	-.227	.028	-.297	-.148	.025	-.046
D	-.010	...	...	-.005	...	...	...	...	...
Mean	-.203	-.148	-.204	-.219	.028	-.297	-.148	.025	-.046

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	...	...	...	...	...	...	...	...	...
B	-.077	-.079	-.298	.058	-.100	-.100	.046	-.006	-.179
C	-.077	-.052	-.298	.058	-.100	-.100	.046	-.006	-.076
D	...	-.010	...	...	...	...	...	...	-.008
Mean	-.077	-.064	-.298	.058	-.100	-.100	.046	-.006	-.123

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	...	...	...	...	...	-.032	...	...
B	.146	.255	.255	.008	-.012	-.029	.385	.101	-.140
C	.146	.255	.255	.008	-.012	-.029	.269	.101	-.140
D	...	...	...	...	...	...	...	...	...
Mean	.146	.255	.255	.008	-.012	-.029	.293	.101	-.140

Allele	F(IS)	F(IT)	F(ST)
A	-.032	-.001	.030
B	-.047	-.037	.009
C	-.047	-.037	.009
D	-.009	-.001	.007
Mean	-.046	-.037	.009

FIS(IK) values

****

Locus: *GOT2*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	-.011	...	...	...	...	-.006	...
B	...	-.005	...	...	...	...	-.015	-.011	-.039
C	.190	-.049	.109	.037	.156	.014	-.162	.177	.226
D	.190	-.074	.145	.037	.156	.014	-.169	.180	.040
E	...	...	...	...	...	...	...	...	...
Mean	.190	-.061	.124	.037	.156	.014	-.161	.172	.122

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	...	...	...	...	...	...	...	...	...
B	-.005	...	-.005	-.006	-.010	-.010	-.010	...	-.008
C	.070	.029	-.120	-.231	-.219	.020	.066	.111	.132
D	.085	.043	-.151	-.288	-.231	.050	.058	.111	.090
E	...	-.005	...	...	...	...	...	...	...
Mean	.077	.035	-.134	-.255	-.221	.034	.061	.111	.109

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	...	...	...	...	...	...	...	...
B	-.005	-.005	-.005	-.026	.385	...	...	-.005	...
C	.006	-.159	-.159	-.190	.159	-.028	.216	.046	.173
D	.021	-.145	-.145	-.290	.137	-.028	.216	.061	.173
E	...	...	...	...	...	...	...	...	...
Mean	.013	-.150	-.150	-.226	.162	-.028	.216	.052	.173

Allele	F(IS)	F(IT)	F(ST)
A	-.009	-.001	.009
B	.034	.045	.012
C	.023	.036	.013
D	.011	.023	.012
E	-.005	-.000	.005
Mean	.018	.030	.013

FIS(IK) values

****

Locus: *MPI*

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	...	...	...	...	...	...	...
B	...	...	...	...	...	...	-.015	-.011	...
C	...	...	-.005	...	...	...	-.020	-.011	...
D	...	...	-.005	...	...	...	-.005	...	...
Mean	...	...	-.005	...	...	...	-.016	-.011	...

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	-.005	...	-.005	...	...	...	...	...	...
B	-.016	...	-.010	...	...	-.026	...	...	...
C	-.021	...	.489	...	...	-.026	...	...	...
D	...	...	-.005	...	...	...	...	...	...
Mean	-.017	...	.239	...	...	-.026	...	...	...

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	...	...	...	...	...	...	...	...
B	...	...	...	...	...	...	...	...	-.005
C	...	...	...	...	...	...	...	...	-.005
D	...	...	...	...	...	...	...	...	...
Mean	...	...	...	...	...	...	...	...	-.005

Allele	F(IS)	F(IT)	F(ST)
A	-.005	-.000	.005
B	-.017	-.003	.013
C	.078	.092	.015
D	-.005	-.001	.004
Mean	.032	.045	.013

FIS(IK) values
****
Locus: GOT1

	Subpopulation								
Allele	1	2	3	4	5	6	7	8	9
A	...	...	...	...	...	...	...	-.005	...
B	...	...	...	...	...	...	...	-.005	...
Mean	...	...	...	...	...	...	...	-.005	...

	Subpopulation								
Allele	10	11	12	13	14	15	16	17	18
A	...	...	-.015	...	...	...	...	...	-.046
B	...	...	-.015	...	...	...	...	...	-.046
Mean	...	...	-.015	...	...	...	...	...	-.046

	Subpopulation								
Allele	19	20	21	22	23	24	25	26	27
A	...	-.015	-.015	-.005	-.015	...	...	...	-.005
B	...	-.015	-.015	-.005	-.015	...	...	...	-.005
Mean	...	-.015	-.015	-.005	-.015	...	...	...	-.005

Allele	F(IS)	F(IT)	F(ST)
A	-.025	-.004	.020
B	-.025	-.004	.020
Mean	-.025	-.004	.020

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